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THE ARTIFICIAL INDUCTION OF SYMMETRICAL CLAWS IN MALE FIDDLER CRABS

PROFESSOR T. H. MORGAN
COLUMBIA UNIVERSITY

THE observations and experiments of a year ago seemed to show beyond much doubt that the young male fiddler crabs pass through a stage in which two large claws are present. The loss of one or the other of these claws is the determining factor leading to the fixed asymmetry of the later stages. The claw that is left becomes the "fiddle," the new claw that replaces the lost one becomes the permanent small claw. The number of cases on which these conclusions rested was not large and the possibility that the induced change was only temporary was not entirely excluded. Therefore I have gone over the same ground again with larger numbers. The results are here recorded. They confirm and extend the earlier findings. *A new and significant result was obtained by removing simultaneously both of the large claws of the young males. Two small claws were regenerated and both remained small throughout later molts.* In other words, if, in the critical stages, the male crab does not become unbalanced by the loss of one of its claws, it loses the power to develop the normal asymmetry.[1]

YEARLING CRABS WITH ONE CLAW ABSENT WHEN COLLECTED

The first young crabs were collected on the beach at West Falmouth on June 12, 1923. These had come ashore

[1] The records and the measurements of the crabs were made by Mrs. A. H. Sturtevant to whose skill and accuracy I am greatly indebted.

in the preceding summer, in July or August, or perhaps later. These were the smallest crabs to be found at this time (June 12). Many of them had one large and one small claw, but there were also 106 male crabs that had lost one claw. Exactly half of them (A) had lost the right claw and half (B) the left one. The former (A) were kept in glass dishes and fed until the next molt when the missing (left) claw reappeared. One escaped, 4 died. Forty were killed after the first molt. The new (left) claw was a small one in all cases. In order to meet the possible objection that these left claws were small because they had had only the time before the next molt to regenerate (an objection of no weight as other results have shown) eight of these crabs were kept alive until the next molt. The left claw remained small (like that of the female) and the right claw remained large. Both showed the normal increase in size that occurs at each molt.

The other half (53) of the collected crabs that had lost the right claw and had a larger left claw were also kept until the next molt (except two that died). Forty-four were killed after the molt. In all, the new right claw was small. Seven were kept until another molt had taken place. The right claw was still small and the left large. One of the seven had, however, dropped its larger claw after the first molt (11 days before the second molt), but this did not affect the outcome since the right claw was small and the left one large. In other words the "set" had taken place.

The preceding results harmonized with previous ones. All these male crabs had presumably reached the stage when two large claws were present and during the winter or spring had lost one of them. Had they remained on the beach they would all have become right or left handed at the next molt as they did in the laboratory.

ISOLATION OF DOUBLE CLAWED MALES

In order to find out how the double clawed young males lost one of their claws and also to find out what

happens in case they do not lose a claw or lose both, fifty-one were isolated, each in a small petri dish with a little sand and water. They were fed every day or two with small pieces of meat (clam). Seventeen died before molting, four escaped; the remainder went through one or more molts and both claws remained large, thus:

7	passed	through	one	molt	then	died
8	"	"	two	molts	"	"
4	"	"	three	"	"	"
2	"	"	four	"	"	"

There were in addition 9 males (of the 51) that dropped one claw. One of these died before molting. Seven dropped a left claw and after the next molt they were found to have regenerated in its place a small left claw. One of these (7) had its large right claw removed while still soft. It replaced this at the next molt by a large right claw. This claw was removed a second time and a large one again came back. The asymmetry had become fixed. Three crabs (of the seven) died before a second molt, three lived through a third molt and two lived to molt once more. No further change in the asymmetry took place.

Of the nine isolated males, one lost the right claw and regenerated a small right claw. From this one the large left claw was removed while it was still soft (after the molt). It regenerated a left large claw at the next molt in place of the large one removed.

Removal of One Claw of Double Clawed Males

From 21 small crabs with two large male claws either the right (A) or the left (B) claw was removed. In ten of these (A) in which the right claw was removed five died before regeneration, five regenerated small claws on the right side. From one of the latter the large left claw was removed eight days after molting. It regenerated a large left claw at the next molt in place of the large one removed. Two of the five crabs were kept alive through another molt. No further change occurred.

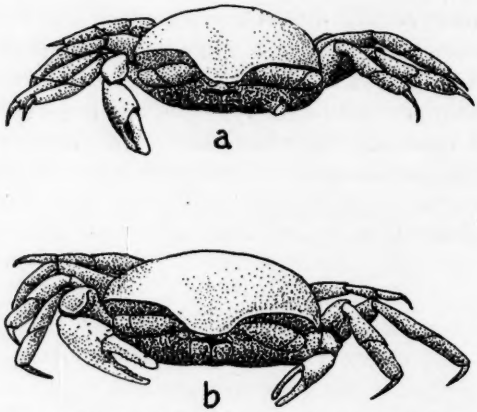

FIG. 1. The upper figure (a) is from a male (four millimeters) with two large claws like the one in the drawing. The left claw was removed. The lower figure (b) represents the same crab after the next molt (four and a half millimeters) when the left claw was replaced by a small claw.

In eleven double clawed crabs (B) the left claw was removed, six died before molting, four regenerated a small left claw. From one of them the large right claw was removed several days after the first molt. A large right claw regenerated in place of the one removed. The asymmetry was fixed.

THE LOSS OF ONE LARGE CLAW FROM OLDER DOUBLE CLAWED MALES

The smallest males with two large claws measure about two and a half millimeters across the thorax. At each successive molt there is a gain of about one millimeter. Amongst the crabs collected with two large claws were some that measured three to four millimeters. The largest isolated males, with two big claws, measured five and a half millimeters. Presumably most of the crabs that were collected above three millimeters and which had equal claws had also acquired them earlier and retained them through more than one molt. The isolated crabs with two large claws molted once or twice before losing a claw. One of these double clawed males lost its left claw

after it had molted and then replaced the one lost by a small claw; five others with big claws molted twice and then lost one regenerating a small one in its place at the next molt (four lefts, one right). It is evident that the male may retain its two large claws for at least two molts and still regenerate a small one if one of them is lost.

Removal of Both Claws from Double Clawed Males

From twenty double clawed males both claws were removed. One died, 19 regenerated both claws which were small. Of these three died before the next molt, nine molted again and both claws remained small, one molted another time and both claws remained small and one molted a third time and remained as before.

These results show that if both large claws are simultaneously removed from double clawed males, they are both replaced by small claws. That the crabs can not subsequently become asymmetrical is shown by later molts. Of course, it is possible that with large numbers, cases might be found when asymmetry appears, but the number suffices, I think, to show that if at this critical stage the symmetry is preserved, as here, by removing both big claws, the crab has lost the opportunity to develop the normal asymmetry of the species.

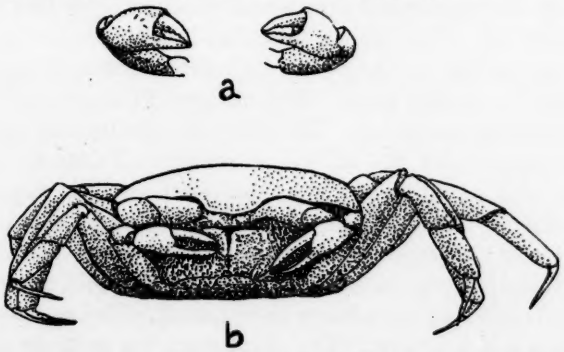

Fig. 2. The upper figure (a) represents the pair of "large" claws removed from a male crab (four millimeters). The lower figure (b) is the same crab after the next molt when two small claws replace the two large ones.

The experiment was carried further as follows. After the two small claws (replacing the two large ones) had appeared, one of them, the right, was removed from three crabs and one of them, the left, from two other crabs. In all cases the results were the same, a small claw appeared after the next molt in place of the small claw removed, and the opposite small claw remained small. In other words, it was no longer possible to produce asymmetry by removing one of the two small claws. Their character had become fixed.[2]

EVIDENCE THAT THE MALE DEVELOPS TWO LARGE CLAWS BEFORE IT DEVELOPS A LARGE AND A SMALL CLAW

In the several collections that were made, especially during the later summer, many very small crabs were collected, that as a rule were smaller than the double clawed male stage. Many of these were kept alive until they molted. Thus 192 crabs (out of 295) remained alive until the next molt. Of these 43 became double clawed males. Since half of these crabs should be females, and since some of them had not, presumably, reached the double clawed stage at the next molt, the appearance of so many (43) double clawed males is a clear indication that most probably all males pass through this stage unless at the preceding stage one of the claws has been already lost. That such a loss may lead to the remaining claw becoming the large one had been shown by my experiments of last year. The number of crabs in those experiments was small. Therefore I repeated the experiment again with the following results. From 44 small crabs with equal small claws the left claw was removed. Twenty-one died before molting; 11 regenerated two small claws, 4 regenerated a right large claw. Of these one died after the molt, one after another molt, one after a third molt and one after a fourth molt. The asymmetry remained in all cases. The results are the same as those

[2] There is a possibility that in both cases the original small claw was removed.

of last year and indicate that the asymmetry may also be induced if at the stage just before the double clawed male stage one claw is removed.

Conclusion

These and the preceding experiments show that the asymmetry of the male fiddler is induced in the young crabs by the accidental loss of one (right or left) claw. If both large claws of the young male stage in which two large claws are present are simultaneously removed no large claws develop but the male remains with two small claws through later molts. The other sexual characters —the abdominal appendages—develop in these small clawed males in the same way as in asymmetrical males. The double clawed males may retain their double claws throughout several later molts, but still retain the power to become asymmetrical if one of the claws is lost. Whether there comes a still later stage when the replacement of a large by a small claw is no longer possible has not yet been shown.

SPECIES HYBRIDS IN CREPIS AND THEIR BEARING ON EVOLUTION[1]

PROFESSOR ERNEST BROWN BABCOCK

UNIVERSITY OF CALIFORNIA

To ascertain and evaluate the natural processes by which the thousands of species of animals and plants, both living and extinct, developed from preexisting species is one of the most interesting and difficult of biological problems. Indeed, there are eminent scholars who assert that the world is little nearer the solution of this great problem than it was a century ago. On the other hand, there are some who claim to have found the solution in a single natural process or method and that it and it alone will solve the whole riddle. However, in view of the evidence at our command, neither of these attitudes is justifiable and, while it may remain for generations yet unborn to comprehend fully and in their true relations the various evolutionary processes, yet there can be no doubt that the utilization of the experimental method of attack is bringing us nearer to a point of vantage from which we can at least survey the realm of nature with more certainty as to interpretation and map out campaigns that will lead ultimately to the desired solution.

Time forbids even a brief discussion of the evolutionary theories generally associated with the names of Lamarck and Darwin. Since Guyer's well-known experiments on the development of antibodies in rabbits that had been injected with serum from fowls inoculated with rabbit eye lens, zoologists especially have taken renewed interest in the Lamarckian view. And some of our plant ecologists hold to the idea that new species arise as the result of the gradual modification of existing species in

[1] Read before the Agriculture Section of the Royal Society of New South Wales, Sydney, Australia, August 10, 1923.

response to environmental stimuli. However, this conception has been generally superseded by the mutation theory of the present day.

De Vries conceived the idea that new species spring full-fledged as it were from their parent species as a result of mutation, a sudden change in the germinal substance. He and other mutationists hold that natural selection plays an important rôle in evolution by eliminating at the very outset the great majority of mutations that are continually appearing in nature. It is now well known that germinal changes resulting in new mutant forms are of various orders of magnitude. It is also generally understood that most, if not all, mutations are caused by some sort of change in the chromosomes. For the sake of brevity I mention only the two general categories of mutations commonly referred to as *point mutations* and *chromosome aberrations.* A point mutation is an alteration of some sort, probably chemical, which changes only a single point or locus of a certain chromosome. A point mutation affects some specific character or characters of the organism, as for example, in the common vinegar fly, color of eyes or length of wings. Such are the mutations which made possible Morgan's brilliant analysis of the heredity of this fly and which in general cause those character differences which distinguish varieties within a given species. A chromosome aberration is an irregularity in the normally regular distribution of the chromosomes during cell division resulting in loss or duplication of whole chromosomes. If we assume that each chromosome contains several or many factors or hereditary units, we should expect the loss or duplication of chromosomes to exert a profound effect upon the soma of the mutant individual, and such indeed is the case. Classical examples are the three inconstant mutants derived by de Vries from Lamarck's evening primrose and now known to have 15 chromosomes instead of 14, which is the normal number for the parent species. Also there is a gigantic form derived from the same parent species, which has 28 instead of 14 chromosomes and

which is known as a tetraploid form, because its chromosome group contains four times the haploid or germ-cell number of the parent species.

In order to make clear the full significance of some of the results I am about to report, it may be well to explain how a tetraploid form might arise. A regular mechanism is present in all sexually reproduced organisms by which the functional germ cells or gametes receive just one half the number of chromosomes present in the somatic cells. The gametic number is, therefore, commonly referred to as the haploid or n-group and the somatic number as the diploid or 2n-group. Most important is the fact that the 2n-group, present in the somatic and primary germ cells, actually consists of *n pairs of homologous chromosomes,* and that in the reduction division preceding gamete formation the n pairs of chromosomes arrange themselves side by side on the equator of the division spindle and then the homologues separate and pass to opposite poles and so become segregated into different gametes. Incidentally, we may note that this separation of the homologues before gamete formation is just the mechanism required to explain Mendelian segregation, if we assume that Mendelian unit factors are carried in the chromosomes and this is now the generally accepted theory. With this mechanism in mind we can readily see how conspicuous mutants might arise through occasional irregularities in the normal mechanism of chromosome distribution. Thus, the failure of one pair of chromosomes to separate in the reduction division would result in one gamete with an extra chromosome which upon uniting with a normal gamete would produce an individual with an extra chromosome. If for some reason the reduction division were to be omitted entirely, gametes containing 2n chromosomes would be formed and reproduction by such gametes would result in a tetraploid or 4n individual. Again, in a tetraploid form, if the reduction division were to be suspended and the gametes containing 4n chromosomes were functional, reproduction would result in an 8n or octaploid individual. We now have good evi-

dence that one of the European species of Crepis with which we are working (*C. biennis* L.) is an octaploid species. It has 40 chromosomes and seems to have been derived from a species having 10 chromosomes (5 pairs) or 5 in the haploid group. Thus, we see that, while point mutations produce new varieties within a single species and while they might in time produce distinct new species having the *same* chromosome number as the parent species, the only mutations capable of producing new species whose chromosome numbers are different from those of the parent species are chromosome aberrations.

Another conception of the manner in which new species originate has recently been championed vigorously by Lotsy and others, who claim that all new species arise through natural hybridization between old species. Here again natural selection accompanied by mere chance must be assumed to preserve the occasional new forms which become established as species. Without going into detailed discussion of the deVriesian and Lotsyan theories of evolution, I will only point out that each has its limitations and that both together comprise only a portion of the whole truth about evolution. Point mutations alone can not explain the origin of species having different chromosome numbers, and chromosome aberrations seem to be so very rare that they could hardly suffice for an explanation of the origin of the thousands of species, genera and families of the sexually reproduced organisms. On the other hand, natural hybridization between species seems to furnish just the necessary means for the origin of new species with different chromosome numbers. But the impossibility of interspecific hybridization having been the sole method of evolution is obvious when we contemplate the simplest forms of life on the earth. The most primitive forms were doubtless incapable of sexual reproduction, so that new forms must have arisen by some sort of mutation. Thus, it appears that the origin of species, either by mutation or by the natural crossing of species, is only a part of the whole evolutionary process or, in other words, that the grand process of evolution depends

on the origin of new species both by mutation and by hybridization, accompanied by natural selection. This hypothesis seems likely to be verified by the results of our Crepis investigations. In fact, the experiments about to be described furnish strong evidence that some new species of flowering plants originate by mutation and others by hybridization.

HISTORY OF THE CREPIS INVESTIGATION

The researches of Morgan and others on the genetics of Drosophila furnished the inspiration for the investigations on Crepis which have been under way at the University of California since 1915. By that time something like 100 mutant characters had been discovered in *Drosophila melanogaster* and these had been shown to exist in four, *and only four,* linked groups corresponding to the four pairs of chromosomes present in this species. There was widespread interest in the Drosophila work at that time, but there was also much doubt and skepticism among biologists as to the generality of the chromosome theory of heredity as developed by the Morgan school. The Drosophila investigations are conducted in laboratories and the flies are reared in milk bottles. It was freely questioned whether the behavior of a species confined in such an artificial environment could be considered typical of other animal species, while the application of the theory to the plant kingdom was still further doubted. In order to make the necessary test with plant material, we were seeking a variable species with very few chromosomes when we found that two European cytologists had discovered that *Crepis virens,* or *Crepis capillaris* (L.) Wallr., as it is now known, has only six chromosomes! Application to various botanical gardens brought seeds of this plant as well as other species of Crepis. We found that *Crepis capillaris,* besides having so few chromosomes, is an annual reaching maturity within three or four months after sowing the seed, that the seeds require only a short rest period, and that the species is so highly variable as to be described in the

floras as polymorphous. We, therefore, set about the genetic analysis of this species and our first comprehensive report will be published in the near future. Meanwhile we found that certain other species of Crepis possess low chromosome numbers and that the genus contains about 170 species which are widely distributed throughout the world. We now have about 50 of these species under cultivation.

Meanwhile Morgan had called attention to the desirability of a crucial test of the chromosome theory of heredity and pointed out that such a test can best be made by crossing species that have been analyzed genetically as we are now analyzing *Crepis capillaris*. Investigators of Drosophila have striven for years to secure interspecific hybrids, but only one such hybrid has been obtained (*D. melanogaster* $\times$ *D. simulans*) and it was completely sterile. Therefore, we soon began experiments on crossing different species of Crepis with the results I am about to describe.

As our familiarity with this group of plants increased our plan of investigation was expanded until now it is organized along three main lines: (1) Genetic analysis of *Crepis capillaris, C. setosa* and other species having but few chromosomes; (2) cultivation of as many as possible of the species in this genus accompanied by taxonomic study and cytologic investigation of chromosome number and individuality, for the purpose of evaluating the ordinarily accepted taxonomic characters in the light of cytologic and genetic data; (3) experiments with species hybrids with three objectives in view—first, to determine genetic relationships; second, to test the chromosome theory of heredity as suggested by Morgan; third, to throw light on the origin of species. I wish to take this opportunity to express my indebtedness to the loyal cooperation of my two colleagues, Dr. J. L. Collins and Dr. Margaret C. Mann, whose keen interest and originality of effort have made possible much of our progress to date, Dr. Collins conducting most of the breeding experiments and Dr. Mann doing all the cytological work,

while the writer is giving special attention to the taxonomic phase of the project.

TAXONOMY OF CREPIS

The plants now referred to this genus present one of the most difficult assemblages from a taxonomic viewpoint. Crepis belongs in the Chicory Tribe of the Sunflower Family and is closely related to Hieracium, so closely, indeed, that there are transitional species which can hardly be separated into the one or the other genus. In Engler and Prantl's Pflanzenfamilien Crepis is comprised of 11 sections or subgenera which differ more or less in characters considered important from a taxonomic viewpoint. For example, in the section Eucrepis, the species all have fruits without beaks, while in the section Barkhausia, all the species have beaked achenes. We are now cultivating species representing all but two of the 11 sections, and shall soon publish a paper on chromosome number and individuality as indicative of taxonomic relationship in these species.

CHROMOSOME NUMBER AND INDIVIDUALITY IN CREPIS

The chromosome numbers of 20 species have now been determined, and we find the following series of diploid numbers: 6, 8, 10, 12, 16, 18, 40. It is very unusual to find such a series within a single genus, the general rule being that a single genus contains only a certain number or multiples of that same number. This at once raises a question regarding the naturalness of the present grouping of species under Crepis. Some of the species studied are very striking in the individuality of their chromosomes. Thus, in *C. capillaris* there are three pairs, one long, one short, and one intermediate in length as was first shown by Miss Digby and Dr. Rosenberg. In *C. setosa* there are four pairs, of which two can easily be distinguished from *capillaris* chromosomes, because the shortest *setosa* chromosome is shorter than the shortest *capillaris* chromosome, while the longest *setosa* chromosome has a peculiar semi-detached portion; but the two

intermediate *setosa* chromosomes can not be distinguished from the intermediate *capillaris* pair. In *C. biennis,* which has forty chromosomes, they are all about the same length, so that they can not be definitely distinguished, but the longer chromosome of *setosa* can be distinguished from the *biennis* chromosomes.

Species Hybrids in Crepis

Thus far we have succeeded in producing six interspecific hybrids as follows (numbers in parenthesis indicate the haploid numbers of chromosomes):

C. capillaris (L.) Wallr. (3) × *C. tectorum* L. (4)
C. setosa L. (4) × *C. capillaris* (L.) Wallr. (3)
C. setosa L. (4) × *C. biennis* L. (20)
C. setosa L. (4) × *C. aspera* L. (4)
C. setosa L. (4) × *C. dioscoridis* L. (4)
C. setosa L. (4) × *C. tectorum* L. (4)

The present paper deals with results from only the first three crosses. In each case the female parent is mentioned first.

Crepis capillaris × Crepis tectorum

There is but little to report on this experiment, inasmuch as thus far we have been unable to induce any first generation plants to grow beyond the cotyledon stage. This is contrary to what was expected because of the close taxonomic relationship between the two species. They are both found in the Eucrepis section and in fact they are quite similar, although sufficiently distinct to be universally considered as good species.

Cytological examination of cells in the root tips of these hybrid seedlings proved that they contained seven chromosomes as would be expected. However, the tissues of the growing point from which the stem should arise were badly disorganized. Apparently, there is something in the germ cells of these two species which makes them so incompatible that normal development of the growing stem is inhibited. As fertilization in the flowering plants is generally accomplished by the union

of one pollen nucleus *without cytoplasm* and the nucleus of an egg cell, it is fair to assume that the germinal incompatibility between *C. capillaris* and *C. tectorum* is due to some relation between the nuclear elements of which the chromosomes appear to be the most important. In short, the abortion of these hybrid seedlings is most reasonably explained as due to inability of the *capillaris* and *tectorum* n-groups of chromosomes to establish a properly organized cell nucleus. This is in harmony with the conception that the chromosome group functions as a single reaction system.

CREPIS SETOSA × CREPIS CAPILLARIS

As these species have been placed in different and very distinct sections of the genus, it might be expected that hybridization would be impossible. Yet it was not only possible to obtain fertile seeds from this cross, but the first generation plants grew vigorously, set seed fairly well when crossed back to either parent, and may even be capable of producing viable seeds when self-fertilized, in spite of the high proportion of sterile pollen grains present in the anthers. The somatic cells contain seven chromosomes, two of which are easily identified as *setosa* chromosomes. The small proportion of viable pollen grains and low degree of self-fertility in the first generation hybrid are readily explained by cytological study of the behavior of the chromosomes during the two cell divisions just preceding formation of the pollen grains. The first of these divisions, in both of the pure species, is the reduction division at which the pairs of homologous chromosomes are separated into different cells and the second is an ordinary mitotic division. In these hybrids, as in species hybrids that have been studied previously by Federley and others, the reduction division is characterized by the fact that none of the chromosomes form pairs (see summary by Täckholm). This would be expected, of course, as none of the chromosomes are homologues, three of them being *capillaris* and the other four *setosa* in origin. The seven chromosomes merely arrange them-

selves in the equatorial region of the division spindle, and then proceed in a hit-or-miss fashion toward one pole or the other. The result must be the formation of gametes containing from 0 to 7 chromosomes in the following proportions, assuming that random assortment of the chromosomes takes place and that all potential gametes mature.

No. of chromosomes in F_1 gametes	Relative frequency of F_1 gametes
0	1
1	7
2	21
3	35
4	35
5	21
6	7
7	1

Now, if we assume that no gametes containing 0, 1 or 2 chromosomes are functional and that of the remainder only those will be functional that contain 3 *capillaris* or 4 *setosa* chromosomes, it is clear that only a small proportion of the gametes would be viable. Furthermore, by backcrossing an F_1 plant to *setosa* and counting the chromosomes of each plant thus obtained, the chromosome numbers in the F_1 gametes will be revealed. Thus far only five plants have been obtained by backcrossing the F_1 to *setosa* and these have chromosome numbers as shown below.

Relative frequency of F_1 gametes	Number of chromosomes in F_1 gametes	*setosa* gametes	resulting zygotes	Zygotes thus far obtained
1	0	4	4	
7	1	4	5	
21	2	4	6	
35	3	4	7	1
35	4	4	8	2
21	5	4	9	
7	6	4	10	2
1	7	4	11	

The two offspring with ten chromosomes in their root tip cells are of special interest. Dr. Mann states that she

can identify in their chromosome groups two pairs of *setosa* chromosomes and one long one from *capillaris*. The identity of the other five chromosomes is still doubtful. These two plants are approaching maturity and when they produce flowers the behavior of the chromosomes in the reduction division will be studied with care as their behavior will show whether they are paired or unpaired. If these 10-chromosome plants contain 8 *setosa* and two *capillaris* chromosomes and are self-fertile, it should be possible by self-fertilizing them to produce two new forms that would breed true, one with 10 and the other with 12 pairs of chromosomes. Except that they would not have been tested as to their ability to maintain themselves in nature, they would really be new species. Even if these two 10-chromosome plants should not give the desired results, in time we shall obtain some that will do so. Thus it is clear that when the chromosomes are irregularly distributed, as in the reduction division of this species hybrid, a means is afforded by which chromosome number may be increased by one or more pairs. And in this way species with chromosome numbers not falling in a series of multiples of a single basic number would be produced. The unusual series of chromosome numbers found on the species of Crepis thus far counted may be explained in this way. Furthermore, if the F_1 hybrids derived from this species cross can be inbred, as now seems likely, it should be possible to observe the effects of one, two or three pairs of *capillaris* chromosomes plus a full set of *setosa* chromosomes on the characters of the plant. This will meet the requirements for a crucial test of the chromosome theory of heredity.

CREPIS SETOSA × CREPIS BIENNIS

This cross also involves the two sections, Eucrepis and Barkhausia, since *C. biennis* has long been considered as close to *C. capillaris* and *C. tectorum*, yet the *setosa* × *biennis* F_1 hybrid is even more self-fertile than the *setosa* × *capillaris* hybrids. This is very remarkable, be-

cause the parent species differ so widely in their chromosome numbers. On this basis it is probably the widest species cross that has ever been made. As I shall soon explain, however, there is a special reason for the higher degree of fertility in this hybrid.

The *setosa* × *biennis* F_1 hybrid has 24 chromosomes in its root tip cells, as would be expected, and two of them can be identified as *setosa* chromosomes. If the behavior of the chromosomes in the reduction division in this hybrid were similar to that which I have described in the *setosa* × *capillaris* hybrid, all the 20 *biennis* and the 4 *setosa* chromosomes would go at random to either pole of the spindle and gametes would be formed containing all the way from 0 to 24 chromosomes. But such is not the case. To our surprise we found that the 20 *biennis* chromosomes form 10 pairs just before the reduction division in this F_1 hybrid, that the 4 *setosa* chromosomes remain unpaired, that the members of the 10 *biennis* pairs separate normally and then after they have moved part way toward the opposite poles the 4 *setosa* chromosomes pass in laggardly fashion and apparently at random toward either pole. This remarkable performance indicates that the 20 *biennis* chromosomes, although brought in by a single gamete, consisted of 10 pairs of homologues and suggests that *C. biennis* is at least a tetraploid species. We now have additional evidence that it is an octaploid species.

The classes of gametes formed by the F_1 *setosa* × *biennis* hybrid, if there is random assortment of the *setosa* chromosomes will conform to the following series:

1 (10 *biennis* + 0 *setosa*)
4 (10 " + 1 ")
6 (10 " + 2 ")
4 (10 " + 3 ")
1 (10 " + 4 ")

If these gametes all survive and unite at random in fertilization, the classes of F_2 combinations produced by self-fertilizing the F_1 hybrid will be:

1	(20	*biennis*	+ 0	*setosa*)	all would breed true
8	(20	"	+ 1	")	
28	(20	"	+ 2	")	those with eleven pairs should breed true
56	(20	"	+ 3	")	
70	(20	"	+ 4	")	those with twelve pairs should breed true
56	(20	"	+ 5	")	
28	(20	"	+ 6	")	those with thirteen pairs should breed true
8	(20	"	+ 7	")	
1	(20	"	+ 8	")	all would breed true

It will be seen that among the F_2 progeny from this species cross 5 types should be found with no unpaired chromosomes and that these are expected to continue as constant races having 20, 22, 24, 26 and 28 chromosomes, respectively. Thus we anticipate the origin of five potential new species from a single species cross by means of self-fertilization of the F_1 hybrid plants. If these new constant races could again be crossed with *C. setosa*, other new types should be produced containing 5, 6, 7, 8 and 9 pairs of chromosomes, provided that we are correct in assuming that *C. biennis* is an octaploid species.

Preliminary observation on about 300 F_2 plants in the rosette stage show that they can be divided into a number of groups according to leaf characters and size of plant, such as the following: *biennis*-like, *setosa*-like, curly-leaved, linear-leaved, pineapple-leaved, broad-leaved ruffled, dwarf (including some of the preceding). One of the most interesting things about this F_2 variability is the possibility that it is due to the difference in number and identity of the *setosa* chromosomes. But there is also the possibility that it is due in some degree at least to recombinations among the *biennis* chromosomes. Recessive factors, which are present but usually masked by dominant allelomorphs in *biennis*, may become manifest in these hybrids. At any rate it seems certain that the new species which we expect to derive in this way will differ sufficiently in morphological characters to be easily distinguished without resort to examination of their chromosomes. But care will be taken to check the chromosome groups of all the distinct F_2 types derived.

By backcrossing the *setosa* $\times$ *biennis* F_1 hybrid to *C.*

biennis it is clear from the F_1 gametic series given above that five types of progeny should be produced having 30, 31, 32, 33 and 34 chromosomes, respectively. Only two such plants have been examined cytologically, but they contain 32 chromosomes in their root tip cells and, what is more important, at the initial stage of the reduction division preceding formation of the pollen grains 15 pairs of chromosomes are present and the homologues separate from each other in regular fashion! This can only be explained by assuming that the wild *C. biennis* is actually an octaploid species, which means that its 40 chromosomes consist of 8 n-groups of 5 each. Whether we shall ever discover in nature the original species with 10 chromosomes (5 pairs), from which *C. biennis* seems to have been derived by repeated duplication, we do not know, but we hope to reproduce it by crossing some of our F_2 plants to *C. setosa.*

Additional reason for assuming octaploidy in the case of *C. biennis* is found in its comparatively large size and vigorous growth. It is much larger than any 5-paired species that we know. If *biennis* is really an octaploid species, the loss of the *gigas* characteristics would not be expected until the 2n condition had been reached. The F_1 hybrid of *C. setosa* $\times$ *C. biennis,* having only 20 *biennis* chromosomes, is as large as *C. biennis* and resembles it closely. We await with anticipation the appearance of the plants that are to have only 10 *biennis* chromosomes.

Resume

Our genetic analysis of *Crepis capillaris* has gone far enough to show that gene or point mutations cause polymorphism within a plant species. But such a process can never bring about the origin of species having new chromosome numbers. Our hybridization experiments with *C. biennis* have produced evidence that species with an increased number of chromosomes may originate through irregularities in the mechanism of chromosome distribution. Our results from crossing *setosa* and *capil-*

laris and *setosa* and *biennis* indicate that new species with different chromosome numbers may originate through hybridization of old species.

Thus we find evidence to support both the mutationist's and the hybridist's conception of the method of evolution. Natural selection among the individuals of new species that have recently arisen through mutation or hybridization must be hypothecated in a state of nature. The inheritance of acquired characters, especially the effects of the environment on plants, *may* act, separately or together with mutation and hybridization, as a slow but definite factor in evolution.

Finally, the results of these experiments indicate the possibility of still greater achievements by the breeders of economic plants. The improvement of crop plants through hybridizing them with related wild species has hardly been begun. Yet in combatting some of the worst diseases and pests of crop plants this is one of the most hopeful lines of attack. It is hoped that plant breeders throughout the world will be encouraged to concentrate both cytological and genetical study along this line, as it gives promise of results of far-reaching importance.

CROSSINGOVER IN THE SECOND CHROMOSOME OF DROSOPHILA MELANOGASTER IN THE F_1 GENERATION OF X-RAYED FEMALES

PROFESSOR JAMES W. MAVOR AND HENRY K. SVENSON
UNION COLLEGE, SCHENECTADY, N. Y.

IN two previous papers the writers have recorded "An effect of X-rays on the linkage of Mendelian characters in the second chromosome of *Drosophila melanogaster*" (Mavor and Svenson, 1923, 1924). It is of interest to determine whether this effect on crossingover, which consisted in an increase of the crossover value, is inherited. The experiment reported here was designed with this end in view. As in the case of the experiments reported in the papers referred to above the X-rayed and control females had the constitution (b pr c/+ + +); that is, they had the genes for black, purple and curved in one chromosome and the genes for the normal allelomorphs of these in the other chromosome. These heterozygous females were mated to males of the constitution (b pr c/b pr c); that is, the males were homozygous for black, purple and curved.

(a) CROSSINGOVER IN THE X-RAYED FEMALES

The X-rayed and control females were the F_1 of two pairs. In the case of those from the first pair "A" six were kept as controls and ten were X-rayed. In the case of the second pair "B" again six were kept as controls and ten were X-rayed. The technique used in these experiments has been described in one of the papers to which reference has been made (Mavor and Svenson, 1924). The X-ray dose was given at a distance of 12.6 cm from the tungsten target of the Coolidge tube with the current at 50,000 volts and 2.5 milliamperes. The time was 20 minutes. On our system of recording dosage this is rep-

resented by 32D. The X-raying was done on November 20 at 7 P. M. We are particularly interested here in the amount of crossingover in the fifth bottles, in which the X-rayed and control females remained from 7:00 P. M., November 26, to 7:00 P. M., November 27, that is, from the sixth to the seventh day after X-raying. It was from among the flies coming out in these bottles that the flies used in the next experiment were taken. The counts of the F_1 in the fifth bottles are given in Table I. In this

TABLE I

COUNTS OF F_1 IN THE FIFTH BOTTLES OF CONTROL AND X-RAYED FLIES

The X-rayed females were treated on November 20 at 7:00 P. M. and immediately mated. The Control females were mated at the same time. The X-rayed and Control females were in the fifth bottles from November 26, 7:00 P. M., until November 27, 7:00 P. M., that is, from the end of the sixth to the end of the seventh day after X-raying.

	No. of parents	Total F_1	Non-cross	Crossover 1	Crossover 2	Crossover 1–2	Percentage Crossingover I	Percentage Crossingover II
Control	6	823	654	26	139	4	3.77	17.38
X-ray	10	180	103	22	47	8	16.67	30.53

table it is seen that for the first region investigated, that between black and purple, the crossover value of the control females was 3.77 and of the X-rayed females 16.67, and that for the second region investigated, that between purple and curved, the crossover value of the control females was 17.38 and of the X-rayed females 30.53. These bottles, therefore, showed an increase in crossingover in the X-rayed females similar to that already recorded in our previous papers. Since females were not bred from all the fifth bottles, Table II is given which shows the crossover values determined by taking only those bottles from which females were bred. This table shows that the bottles from which the flies were chosen gave almost exactly the same crossover values as those in Table I, obtained for all the X-rayed and control flies in the fifth bottles.

TABLE II

COUNTS OF THE F_1 IN THE ACTUAL BOTTLES FROM WHICH THE FEMALES WHOSE F_1 ARE RECORDED IN TABLE III WERE TAKEN

Four out of the 6 controls and 6 out of the 10 X-rayed females produced the F_1 recorded in this table.

	No. of parents	Total F_1	Non-cross	Crossover 1	Crossover 2	Crossover 1–2	Percentage Crossingover I	Percentage Crossingover II
Control	4	417	332	11	71	3	3.36	17.7
X-ray	6	157	90	20	41	6	16.6	29.9

(b) CROSSINGOVER IN THE F_1 OF THE X-RAYED FEMALES

In order to test whether this increased crossover value was transmitted to the next generation, females from among the F_1 of the X-rayed and control females recorded in Table II were bred. Females of the same constitution as their mothers, that is, of the formula (b pr c/+++), were chosen and mated to males of the formula (b pr c/b pr c), that is, of the same constitution as their fathers. The same cross was, therefore, made in the case of the F_1 of the X-rayed and control females as was made when the X-rayed and control females were mated.

TABLE III

COUNTS OF THE F_2 OF X-RAYED FEMALES

These flies are the F_1 of virgin females of the constitution, b pr c/+++ from among the F_1 produced in the fifth bottles of the experiment and recorded in Table II. The parents, the crossingover in which is recorded in this table, remained in the first bottles from December 6 to December 9, and in the second bottles from December 9 to December 18.

Bottle	No. of Parent	Total	Non-cross	Crossover 1	Crossover 2	Crossover 1–2	Percentage Crossingover I	Percentage Crossingover II
				Control				
1st	5	325	277	10	37	1	3.38	11.64
2nd	5	456	378	14	60	4	3.94	14.01
Total	5	781	655	24	97	5	3.72	13.07
				X-rayed				
1st	14	654	521	18	105	10	4.28	17.58
2nd	14	821	719	21	73	8	3.53	9.88
Total	14	1475	1240	39	178	18	3.86	13.29

The results of breeding the F_1 of the X-rayed and control flies are shown in Table III. The flies were passed through two series of bottles and the crossover values are given separately for the first and second bottles. The total crossover values obtained by adding the counts of the first and second bottles show no perceptible difference between the crossover values for the F_1 of the X-rayed females and the F_1 of the control females. In the case of the F_1 of the control females the crossover value for the black to purple region was 3.72 and for the purple to curved region 13.07. The corresponding values for the F_1 of the X-rayed females were 3.86 and 13.29. In the first region, black to purple, no significant difference is seen in the crossover values obtained for the first and second bottles. In the case of the second region, purple to curved, the results are not so uniform, a noticeable difference being seen between the first and second bottles of the F_1 of the X-rayed and the F_1 of the controls. In this case the crossover value for the F_1 of the X-rayed in the first bottles, 17.58, is greater than that of the F_1 of the controls, 11.64, and the crossover value of the F_1 of the X-rayed in the second bottles, 9.88, is less than that of the controls, 14.01. It is doubtful, however, whether these differences when compared with the probable errors of the differences are significant. The difference divided by the probable error for the first bottles (F_1 of X-rayed compared with F_1 of control) is 3.99, and the same quantity for the second bottles is 3.05.

The conclusion to which this experiment leads is that the effect of X-rays on crossingover in the second chromosome is probably not transmitted, at least in anything like its original intensity, to the F_1 of the X-rayed females. This is in agreement with the results of Plough (1917) on the effect of temperature on crossingover in the second chromosome. He found (p. 166) that the effect of temperature, consisting in an increased crossover value, was not transmitted to the F_1 of the heat-treated females. The objection may, however, be raised that the F_1 females which were bred did not arise by crossingover

in the X-rayed females, having the same constitution as their mothers so far as concerned the characters used. Since crossingover did not occur in the eggs which gave rise to the F_1 tested, such eggs may have been unaffected by the X-rays and therefore the individuals arising from them would not in any case be expected to transmit an X-ray effect. The experiment ought, therefore, to be repeated, using F_1 of the X-rayed and control females which were crossovers, although of course here again the results would not be entirely free from the same objection, since even in these only a proportion of the crossovers owe their origin to the effect of X-rays.

The pressure of other work makes it unlikely that the writers will be able to repeat this experiment in the near future in a form which would make it conclusive so far as the transmission of the effect of the X-rays is concerned. The results are therefore submitted as they stand and the conclusion is drawn with the reservation considered.

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COUNTED GRAIN POLLINATIONS IN MATTHIOLA

R. SNOW

THE DEPARTMENT OF BOTANY, UNIVERSITY OF OXFORD

THE remarkable phenomena of inheritance of factors for flower form and plastid color in the Stock, Matthiola, as investigated by Miss Saunders (1911) are generally well known, and have already led to the suggestion that half of the pollen grains may fail to function. (*Cf.* Frost, 1915.) But it may be as well to recall such of the facts as here concern us.

Single stocks may be genetically of two kinds, pure singles or ever-sporting singles. The former breed true to singleness; the latter, when selfed, give a progeny of singles and doubles in the proportion of about five singles to six doubles. Of these, the doubles are sterile, and the singles are ever-sporting like their parent. This behavior is further explained by the results of crosses. The ever-sporting type is heterozygous for singleness (a dominant over doubleness), but whereas it forms ovules carrying either factor, its pollen all carries doubleness. If, then, the ovules are formed in the proportion of five single to six double-carrying, the observed results will follow. A further complication is that the factor or factor-complex for singleness does not occur in the same condition in the ever-sporting type as in the pure single. For in the cross, pure-single by ever-sporting, the heterozygote thus formed is not ever-sporting, but gives an F_2 with nearly a normal ratio of three singles: one double, thus showing that its single-carrying pollen is formed normally. Thus, the peculiar behavior of the ever-sporting type must be due to its singleness-complex, and for present purposes we may most simply make use of the notation used by Frost,

Pure single = SS
Ever-sporting = S's
Double = ss

The interest of the situation then lies in the fact that the distribution of factors amongst the ovules appears to be different from that in the pollen of the same plant. Such cases have been considered as due to "somatic segregation," but it is not clear how this conception would be applicable to the present case, even if such a process were possible. For we have here not the separation of two allelomorphs that thenceforward persist independently, as commonly understood by segregation, but rather the apparent loss of one allelomorph, the factor for singleness, on the pollen side of the flower.

Miss Saunders speaks indeed of an "elimination" of the singleness-complex before meiosis (1920, p. 184), and this could be reconciled with current views of chromosome mechanism and segregation at meiosis, if interpreted in the sense of a regularly recurring loss mutation somewhere in the cell-divisions leading to pollen formation. But even this means introducing a new hypothesis, which could be avoided if it could be supposed that both sorts of pollen are formed, but that at some stage the S′ pollen fails to function, through the action of a gametic lethal. Accordingly, it was suggested to the writer by Mr. J. B. S. Haldane, who had devised a factorial scheme explaining remarkably the numerical results in inheritance of flower form and plastid color, but involving gametic lethals, that he should attempt to find out whether such were present in the pollen. The results so far obtained reveal a peculiar situation of some kind in pollen formation, and may be of interest as indicating a method of genetic research of which surprisingly little use has been made.

Under the microscope the pollen of both types of plant appears nearly all sound and uniform. It may conveniently be examined by mounting in potash and pressing on the cover-slip, when the contents will slip intact out of the spore-coats. There are a very few small badly formed grains. In tap water many of the grains germinate, but the tubes mostly burst soon afterwards. In 20

per cent. or 30 per cent. sucrose, almost all the grains germinate, and the tubes begin to grow. But since failure of certain pollen grains might occur at any stage of the processes between pollination and fertilization, it was decided to compare the pollen of pure singles and ever-sporters by counting out definite numbers of grains, placing them on the stigmas and determining the ratios of seeds obtained to grains applied, in the two cases.

The method was as follows: Glass needles were made, long and straight, by twice pulling out a solid glass rod in the blow-pipe flame—the second time with the flame very small. The pollen, of which each grain measures 30μ by 17μ, was spread thinly on a dry glass slide. Needles and a microscope were taken out to the flower bed. A needle was swept through the pollen on the slide, and then examined under the low power of the microscope. The grains taken up by the needle were thus easily counted, and if not too many were applied to the two lateral tufts of stigmatic hairs. The needle was then reexamined to make sure that it had given up all its grains.

The pollen was taken from anthers that had just opened in the bud. The buds of the ovule-parent were destaminated on the last day before the anthers would have opened, and pollinated when the stigmatic hairs looked most receptive—about 48 hours later.

The plants employed were grown from seeds of several races kindly sent by Miss Saunders to the Oxford botanical department. The number of grains placed on the stigmas of each flower was between 20 and 30; since the ovaries contain from 35 to 60 ovules, these would always be in excess. Had such low ratios of seeds to grains been anticipated, larger numbers of grains would naturally have been used. For ovule parents, ever-sporting plants were used throughout, as likely to give more critical results in the next generation. Care was taken that neither kind of pollen should be in any way favored as against the other. Pollen was taken from races of

equal vegetative vigor. Different flowers on the same raceme of the ovule-parent were usually pollinated alter-

TABLE I.

Pollen of pure singles		Pollen of ever-sporters	
d-cream by no-d-cream		d-cream by d-sulphur-white "R.H.K."	
Grains	Seeds	Grains	Seeds
22	9	24	0
22	0 (died)	24	0 (died)
22	1	26	0
23	4	24	0
25	2	20	0
20	0	20	1
23	0 (died)	23	0
25	9	20	0 (died)
20	2	22	0
26	2	22	0 (died)
24	0	28	0
24	0 (died)	28	0
27	1	20	0
22	1	23	0
Total 325	31	324	1

TABLE II.

d-sulphur-white CK by no-d-white		d-sulphur-white CK by d-sulphur-white CK	
Grains	Seeds	Grains	Seeds
28	8	24	1
25	0	25	0
23	1	24	0 (died)
		22	0 (died)
		25	0
d-sulphur-white CK by no-d-cream		d-sulphur-white CK by d-sulphur-white R.H.K.	
26	0 (died)	22	0
22	0 (died)	22	0
25	3	24	1
23	0		
23	2		
d-sulphur-white R.H.K. by no-d-cream		d-sulphur-white R.H.K. by d-sulphur-white CK	
25	3	23	0 (died)
d-red (family "19") by no-d-white		d-red (family "19") by d-sulphur-white CK	
26	0	24	0 (died)
24	1	25	0
26	0 (died)	23	0
		21	0
Total 296	18	304	2

nately with pollen of each kind, and all stigmas were, so far as possible, pollinated under the same conditions. The pollinations of Table 2 were made earlier in the year, when the routine had been less standardized. The work was much hampered by the cold and rainy summer of 1922.

The results are given in the following two tables. After pollination the ovaries in a few cases died, but usually persisted and grew to 20 mm or more even if they had set no seed. Ever-sporters are described as "d" and pure single as "no-d" plants.

Thus, in all, 621 grains of pure single pollen, distributed amongst 26 ovaries, gave 49 seeds, whereas 628 grains of pollen of ever-sporters, on 27 ovaries of the same ovule-parents, gave three seeds.

The ratios of seeds to grains were thus extremely low (1 to 12.7 for the pure singles), and possibly do not represent the chances of success of a pollen grain in normal mass pollination. For there may have been something unfavorable to seed-setting in the pollinations with small numbers of grains, either through ignorance of some essential factor, or possibly because the style and stigma may need to be brought into some favorable physiological condition by the stimulus effect of pollination with larger numbers. If the ratios obtained above held good, then in the case of "d" pollen, it would need about 8,000 grains to enable one flower to set a fruit nearly full of seed (as the sulphur-whites were found to do when left to self-pollinate naturally). This seems a lot, but calculations based on measurements of grains and of pollen sacs showed that each stamen contains about 124,000 grains, and each flower therefore 744,000. But the limiting factor will be the seating room on the stigma. However this may be, there still remains the question, why so great a difference between pollen of the two types. This might be due merely to a peculiarity of the two sulphur-white races used as pollen parents. But as against this, when selfed they set fruits nearly full of seed, while one of

them (RHK) was a strong-grower and a good match for the no-d-cream race. It is hoped to make further experiments, if necessary facilities can be obtained, with pollen from other "d" races, and also with "no-d" races as ovule parents.

But if the difference were really due to the different constitutions of "d" and "no-d" plants, then certain conclusions would follow. Since the difference is too great (being much more than 50 per cent.) to be due merely to elimination of S′ pollen in the S′s plant, the s pollen must also be weakened. But since, as mentioned above, in the Ss hybrid from the cross SS × Ss, s pollen is known to be formed in nearly the normal proportion, it follows that its partial deficiency in the S′s plant must be due to the constitution of the parent plant (unless to a direct lethal effect of the S′ pollen upon it).

Whether the total elimination of S′ pollen from the S′s plant is partly dependent on the parental constitution, or due entirely to a gametic lethal, could similarly be determined if it were known whether or not s′ pollen is formed in the s′S hybrid, from the cross S′s × SS. Experiments on this point are in progress.

For another method of counted grain pollination reference may be made to the work of Goodspeed (1918), on Nicotiana.

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THE PROBLEM OF PATTERN IN ORGANISMS[1]

II. THE PHYSIOLOGICAL GRADIENTS

PROFESSOR C. M. CHILD
THE UNIVERSITY OF CHICAGO

THE PHYSIOLOGICAL GRADIENTS AS FACTORS OF PATTERN

MOST organisms show some indications of a physiological axis or polarity. This means that not only the spatial, morphological order of parts and organs, but also the sequence of events in development and the physiological relations between parts are referable to certain directions in the protoplasm or cells composing the organism, or, geometrically speaking, to a line, the axis. With this axiate order is associated some sort of symmetry, either radial or bilateral, or some combination or modification of these. This means that in addition to the major or polar axis, we can distinguish other minor directions or lines, to which certain features of the order are referable. In fact, so far as its spatial characteristics are concerned, axiate pattern is referable to a tri-dimensional system of coordinates, in which the major or polar axis always differs in significance from the other two, while these may be alike and either indefinite or definite in direction, as in different sorts of radiate pattern, or they may differ from each other, as in bilateral pattern. In some organisms a simple axiate pattern persists throughout life; in others it is at first simple and becomes multiple, or minor axiate patterns of parts or organs arise in it in the course

[1] This paper, essentially in its present form, was written and accepted as a contribution to the proposed Williston Memorial Volume. Publication of that volume having proved impossible, the paper is dedicated to the memory of Professor Samuel Wendell Williston, both as an expression of personal regard and as a record of the purpose for which it was originally intended. The paper represents in considerable part the combined subject-matter of several addresses given at the University of Washington, the University of Wisconsin, the University of Cincinnati and elsewhere.

of development; again, the original pattern may be variously altered or modified during development, or it may even be replaced by another of different sort. In the higher animals the axiate pattern becomes exceedingly complex, the axes of parts, organs and cells showing all possible directions with respect to the primary axes.

Various lines of investigation show that the physiological axis in its simplest, most primitive condition is a gradient in physiological state, primarily quantitative in character, and involving a gradation in rate of the fundamental metabolic reactions, as well as in protoplasmic conditions. These gradients have been called metabolic, axial or physiological gradients. So far as investigation has gone, they have been found to be characteristics, not only of the major or polar axes of organisms, but of the symmetry axes as well, and also of the axiate patterns of organs and parts. Localization and differentiation in development occur in a definite relation to these gradients, the high end of the polar gradient, for example, becoming the apical end or head and the other organs along the polar axis arising at different levels of the gradient. In the bilateral invertebrates, so far as examined, the high region of the symmetry gradients becomes the median ventral, in the vertebrates the median dorsal region. In short, the system of axes to which axiate pattern is referable appears to be primarily a system of physiological gradients in the protoplasm. We may say that each point in the organism is determined and characterized by its position in the gradient system.

These gradients, however, are not necessarily present throughout the protoplasm of the body. The evidence indicates that they appear primarily in the superficial regions of the cell or multicellular body. In at least many protozoa and plant cells the gradients exist only in the superficial regions, and it is only these regions which show definite axiate pattern. But where definite internal organs with an axiate pattern exist, these also show physiological gradients, though both the original and the final

relations of these to the primary gradients differ in different organisms and according to the course of development. In many of the simpler organisms the primary gradients persist throughout life, but even in such forms they may undergo modification and complicaton in various ways. The primarily quantitative gradation usually gives rise in the course of development to a series of qualitative differences at different levels and the original gradient may disappear, leaving the effects and relations determined by it.

The existence of the physiological gradients is indicated or demonstrated in many different ways. A method of considerable value, particularly for the simpler organisms and earlier stages of developmental stages, is the susceptibility method. Extensive experimental investigation has shown beyond question that a general relation exists between physiological or metabolic condition in protoplasm and susceptibility to the toxic action of certain ranges of concentration or intensity of at least many if not all external agents. To certain ranges of concentration or intensity above the limit of tolerance or acclimation, susceptibility varies directly with rate of fundamental metabolic reactions. In such concentrations or intensities the higher levels of a physiological gradient are earlier, or more completely inhibited in growth and development, or die earlier than less active levels. In certain lower ranges of concentration or intensity which permit acclimation or recovery after temporary exposure, the higher levels of a gradient undergo the processes of acclimation or recovery more rapidly or more completely than lower levels. In other words, the more active protoplasm is more susceptible to extreme conditions and more capable of adjusting itself to less extreme conditions than the less active.

The susceptibility method may be used in various ways. The differential susceptibility along a physiological gradient may be indicated by differences in survival time, or, under somewhat less extreme conditions, by dif-

ferences in degree of inhibition of growth or development or motor activity, and under still less extreme conditions, or after temporary exposure, by rate or degree of acclimation or recovery. Through the differential susceptibility of different levels of a physiological gradient it is possible to alter the course of development in certain definite, predictable ways, determining great differences in size and proportions of parts, or even their presence or absence. Unquestionably a large proportion of teratological forms, appearing in nature as "accidents," result from differential susceptibility at different levels of the physiological gradients of developmental stages.

The existence of the gradients is also indicated by differences in rate of penetration of various substances, *e.g.*, vital dyes, by differences in enzyme activity, in rate and amount of reduction of $KMnO_4$, and in electrical potential. In certain forms it has also been possible to demonstrate directly that the gradients are gradients in rate of oxygen consumption and CO_2 production. All these physiological differences at different levels of the gradients disappear when the organism is killed or soon afterward.

In many forms the gradients are indicated by structural gradations along the axis, such as a gradation in cell size, as in many animals and plants the protoplasm-yolk gradation in many animal eggs, the degree of vacuolation in many plant embryos, etc. The gradients also appear in the rate and sequence of development along the axes. In axiate animals development at first proceeds most rapidly at the high end of the polar gradient, which becomes the apical end or head, and in the high region of the symmetry gradients, the median ventral region in the bilateral invertebrates, the median dorsal in vertebrates.

The primarily quantitative differences at the different levels of a physiological gradient serve as the basis for determining the qualitative differences which arise in differentiation. This may occur in various ways. For example, a difference in the relation between intake of nu-

trition and the rate of oxidation at different levels may determine the accumulation of certain substances in the protoplasm at one level and their absence at another. In many yolk-bearing animal eggs the yolk appears chiefly or only at the lower levels of the polar gradient where oxidation is least rapid. Again, differences in concentration of certain substances at different levels may determine different reactions and different products and so initiate differentiation. Moreover, in so complex a system as protoplasm differences in state of colloids, enzyme activity, electrolytic dissociation, water content and various other factors at different levels of a gradient may also be factors in the initiation of differentiation. As soon as differentiation begins, definite and orderly chemical correlation becomes possible and plays a part in further differentiation.

DOMINANCE AND SUBORDINATION IN RELATION TO THE GRADIENTS

Experimental investigation has shown that a relation of dominance and subordination exists between different levels of a physiological gradient. In general, any level is to some extent dominant over lower levels and is dominated by higher levels and the high end of the gradient dominates all lower levels within a certain distance. This means that a given level of the gradient affects physiologically lower levels of the gradient to a greater degree than they affect it. This is merely a special case of the general rule that a more active region of protoplasm affects less active regions more than they affect it. All the evidence at hand indicates that this relation of dominance and subordination depends primarily, not upon the mass transportation of substances from one level to another, but upon the transmission of the energy changes which constitute physiological excitation. It depends essentially upon the fact that a higher level of the gradient behaves with respect to a lower level like a region of excitation with respect to an unexcited or less excited

region. Dominance appears, not only in the functional relations along an axis, but in the developmental relations as well. It has been demonstrated experimentally for *Planaria* and some other forms that localization and differentiation of organs along the polar axis are physiologically determined primarily in the direction from anterior to posterior and not in the opposite direction.

Physiological dominance is limited in range and its range varies with the activity of the dominant region and the condition of the protoplasm through which the transmitted change passes. When we decrease experimentally the metabolic activity of a dominant region, *e.g.*, the growing tip of a plant, the apical end or head region of an animal, its dominance decreases in range, or may disappear, if the decrease in activity is sufficient. Under these conditions changes may occur at lower levels which did not take place as long as the dominance persisted. In plants, for example, new buds may appear, or buds previously inhibited may grow, and in the simpler animals, where differentiation has not progressed too far, similar reproductive processes may take place. These are cases of physiological isolation. In general, physiological isolation has been shown to occur in four ways: By increase in size of the body beyond the limit of dominance and consequent isolation of parts most distant from the dominant region; by decrease in activity of the dominant region; by blocking the correlative factor in its passage through the protoplasm; and finally by increasing the activity of a subordinate part to such an extent that it is no longer subordinate. It has also been shown that physiological isolation is the primary factor in at least many processes of agamic reproduction and reduplication of parts, and it is probably concerned in all such processes.

Physiological dominance is, in fact, one expression of the physiological gradient. The attempt has been made recently to show that a very direct physiological continuity exists between the physiological gradients and the structural and functional relations of the nervous system

in animals. To sum up, the physiological gradient in its simple form, both as regards the differences in physiological condition at different levels and the relation of dominance and subordination, shows all the characteristics of an excitation-transmission gradient. In fact, the only marked difference is that the physiological gradient is relatively permanent as compared with the ordinary excitation-transmission gradient. But whether this similarity has any real significance can be determined only by investigation of the origin of the physiological gradients.

THE ORIGIN OF THE PHYSIOLOGICAL GRADIENTS

Our knowledge along this line is still very incomplete, but various suggestive and some very conclusive facts are at hand. In the first place, physiological gradients are determined in various organisms by a quantitative differential in the action of an external factor. In such cases the external factor either localizes a region of high activity, essentially a region of excitation, and the gradient arises from this, or the differential action on different regions of the protoplasm may directly determine the gradient. In the eggs of certain species of the alga *Fucus* the physiological axis, which has been found to be a gradient, is determined by the differential action of light, the most strongly illumined region becoming the apical pole of the plant. In the *Equisetum* spore the physiological axis is similarly determined, and the determination of dorsi-ventrality in plants by the differential action of light is a familiar fact.

In pieces or cell-aggregations of the simple animals, *e.g.*, sponges and hydroids, new polarities may be determined by differences between a free and an attached surface, the free surface becoming the apical and the attached, the basal pole. In other cases an apical pole and so a new axial gradient may be determined directly by localized injury which gives rise locally to high metabolism and rapid growth and so determines the localization

of a dominant region. According to Lund a new physiological polarity may be determined in hydroids by means of the electric current and the facts show that in such cases a new physiological gradient is determined. In still other cases, such as the localization of adventitious buds in certain plants and the development of new axial gradients in hydrozoan embryos and pieces after experimental obliteration of the preexisting axes slight chance differences in activity between different cells or cell groups are sufficient to determine the new dominant regions and so the new gradients and the whole course of development of new individuals.

In various forms among the lower invertebrates the free pole of the growing egg becomes the high end of the polar gradient and the apical or anterior end of the embryo. The actual determining factor in these cases is unknown, but it may be a differential in oxygen supply or in CO_2 removal or both. In the higher animals, where the respiratory exchange is effected chiefly through the blood, the circulatory relations of the growing egg appear, at least in some cases, to be a factor in determining its gradient. In most plant eggs also the polarity is apparently determined by a differential relation to the parent body. Further experimental work is necessary for the determination of the particular factors of the environmental relation which are concerned in the origin of egg-polarity.

As regards the origin of symmetry in development, particularly bilaterality, our knowledge is still less complete, except in the matter of its relation to light or other external factors in plants. It is possible to obliterate symmetry experimentally and to determine different sorts of symmetry in the same protoplasm and a symmetry gradient may be substituted experimentally for a polar gradient, but as regards the origin of symmetry in embryonic development in animals we know but little. In certain cases it is perhaps determined by the spermatozoon, in others perhaps by the position of the matura-

tion spindle before it moves to the apical pole or of the first cleavage spindle or some other division.

In some cases of agamic reproduction the physiological gradients of the new individual are determined anew in response to local conditions, but in others the old gradients persist as such through the reproductive process and the axes of the new individual are determined by these gradients. In such reproductive processes the dedifferentiation and acceleration of metabolism involved in the reorganization of the part into a new individual bring the gradients up to the physiological levels characteristic of other individuals. In these cases the gradients, and therefore the axial relations, are directly inherited as gradients from the parent individual. To what extent such inheritance of the axial gradients from earlier cell generations occurs in eggs is uncertain, but that it may occur is of course possible. It should be pointed out, however, that such persistence or inheritance does not involve Lamarckian assumptions. There is in such cases no transmission or transfer of something from the body to the germ, but merely a persistence of certain physiological conditions through several or many cell generations. When the planarian body undergoes fission or is cut into pieces, the axial gradients usually persist and the axes of the new individual are the same as those of the individual from which it came. If inheritance of axial gradients in eggs occurs, it is likewise simply a persistence of physiological conditions previously determined and in no sense a Lamarckian inheritance. On the other hand, the gradient conception may provide a physiological basis for certain phenomena which are often interpreted in Lamarckian terms.

Whether or not the axial gradients persist through reproduction, the evidence indicates that they arise in the first instance as a response to a differential action of a factor external to the protoplasm concerned. The external factor determines directly or indirectly a high rate of activity in some region or a differential in rate in

different regions and the excitatory changes, transmitted with a decrement from the region of highest activity thus determined, give rise to an excitation-transmission gradient. If the action of the external factor continues for a sufficient length of time, more or less permanent changes in protoplasmic conditions are brought about, corresponding to the different degrees of excitation at different levels. These changes constitute the substratum for a more or less permanent gradient and undoubtedly determine its further development after the external factor has ceased to act. The physiological gradient then originates essentially as a differential excitation in response to action of an external factor, but when once established through the occurrence of protoplasmic changes which are not readily reversible, its further development is independent of the external factor, but is of course determined by the specific hereditary constitution of the protoplasm in which it exists.

According to this conception axiate pattern originates as an excitation-transmission pattern which determines more or less permanent protoplasmic changes and so becomes the basis of the developmental pattern. The relation of dominance and subordination is primarily the relation between higher and lower levels of the gradient and constitutes the basis of the functional relations which attain their highest development in the nervous system. As differentiation in relation to the gradient occurs, it affords a basis for orderly and definite chemical or transportative correlation, and this beomes increasingly complex and important as differentiation progresses and channels of transportative communication develop.

The Problem of Surface-interior Pattern

The cell is primarily an organism, though it may be integrated with other cells into an organismic pattern of larger scale. There are also certain organisms, *i.e.*, bacteria, which appear to be even simpler than cells of the

usual type. In most unicellular organisms and even in some of the still simpler organisms axiate pattern undoubtedly exists, but apparently the most general feature of pattern in these simple organisms is the completely radial or spherical symmetry with differences between surface and interior. This surface-interior pattern is apparently a more generalized and more primitive pattern than the axiate, in that the only differences are those between the surface as a whole and the interior. Any mass of protoplasm exposed to the action of environmental factors must acquire some sort of surface-interior pattern, since conditions at the surface are different from those in the interior. It seems probable that surface-interior pattern is not fundamentally different from axiate pattern, except that in the former the differential is between the surface as a whole and the interior, while in the latter a differential between different parts of the surface exists. But since we can reach the interior only through the surface, it is difficult to determine whether a physiological gradient, originating in excitation-transmission relations from surface to interior, is present in surface-interior pattern, or to what extent the material exchange between the protoplasm and the external world is concerned in determining this sort of pattern.

The cell and at least many organisms simpler than cells apparently represent primarily surface-interior pattern. The localization and differentiation of the nucleus was probably originally determined by conditions in the interior of the mass of protoplasm. It is, in fact, difficult to conceive how the nucleus as a definite organ could have arisen in any other way. Moreover, there is no reason to believe that the persistence of the nuclear substance from one cell generation to another and through varying conditions is entirely independent of the conditions in the interior, as distinguished from the surface of the cell.

The presence of axiate pattern does not mean the disappearance of surface-interior pattern. In fact, surface-

interior pattern exists in all organisms, whether unicellular or multicellular. Axiation and symmetry represent merely new differentials superimposed on the more general surface-interior differential. The surface-interior pattern is of course complicated and modified by the presence of an axiate pattern, but still exists and must exist in some form in any mass of protoplasm exposed to an environment, and all living things are so exposed. It is the most general and most primitive organismic pattern, and at least certain of its features are obviously not inherent in the protoplasm, but are directly dependent on exposure to the action of external factors. For example, the appearance of a plasma membrane or an ectoplasmic layer is a direct result of exposure of protoplasm to environment. This is perhaps the most general structural characteristic of organismic pattern and the first step in organization, and if this is dependent upon external factors, the preformistic conception is discredited at the outset.

As in the case of axiate pattern, the particular kind of surface-interior pattern which arises in any given case depends primarily upon the specific hereditary constitution of the protoplasm concerned. The relation to external factors merely determines that surface-interior pattern of some sort shall be present. The character of surface-interior pattern may differ widely in different protoplasms, even in the same environment, but this means, not that the pattern is inherent, but rather that the potentialities of behavior in a given sort of organismic pattern are primarily dependent on the nature of the material on which the pattern is imposed.

Organismic Pattern and the Chromosomes

Whether or not we regard cell pattern as primarily of external origin, recent investigations and current theories make it necessary at least to inquire concerning the significance of the chromosomes for organismic pattern and particularly for the pattern of multicellular organ-

isms. Certain lines of recent work in cytology and genetics have led to the postulation of definite, more or less stable chromosome patterns, differing for each chromosome, and some biologists believe that the totality of chromosome patterns in the nucleus of the germ cell represents in some way, or constitutes the basis of the organismic pattern which appears in development of the individual.

It is, however, a well-established fact that in general every cell of the multicellular organism possesses the full complement of chromosomes and therefore supposedly the complete nuclear pattern of the fertilized egg. Nevertheless, different cells or cell groups become different and different kinds of organismic correlation arise between them. Recognition of this fact led Boveri years ago to admit that local conditions, rather than the nuclear pattern, must be responsible as activating or determining factors for the origin of these differences. In other words, Boveri held that organismic pattern must be of external origin. More recently, however, this question has either not been considered, or the fact has not been recognized that cells which are originally alike can not of themselves become different. Loeb, in "The Organism as a Whole," merely states the problem when he says that "the egg is the embryo in the rough" and does not attempt to show how the egg has attained this condition. Similarly, Morgan, in a recent discussion of the organism as a whole, does not account for the fact that cells, which are originally alike as regards nuclear pattern, become different in orderly and definite ways.

As Conklin, F. R. Lillie and others have shown, various eggs possess more or less definite cytoplasmic patterns, but if we maintain that these patterns are determined by the nuclear or chromosomal pattern and that the pattern of the multicellular organism results from the distribution of the components of this pattern among different cells in development, we have merely formulated the problem of organismic pattern in the preformis-

tic terms of nuclear pattern, and the problem of nuclear pattern remains. Moreover, this conception involves still another difficulty. If nuclear pattern is the fundamental factor in determining the cytoplasmic pattern and if the cytoplasmic pattern of the egg represents the primary pattern thus determined, as this conception apparently assumes, we should expect each cell which arises from the egg with a full complement of chromosomes gradually to approach the cytoplasmic pattern of the egg, even though different cells originally received different components of the egg cytoplasm. In short, since they all possess the same nuclear pattern, the different cells should become more and more alike, instead of more and more different. But, since they do become more and more different, it must be assumed, either that cytoplasmic pattern, once established, may continue to exist independently, or in spite of nuclear pattern, or else that such cytoplasmic pattern may influence and alter nuclear pattern. Either of these assumptions amounts to abandonment of the original hypothesis of nuclear or chromosomal pattern as the determining factor. It is evident that, even when we start with the cell conceived in terms of the theories of nuclear pattern, something more is necessary, *viz.*, the action of an external factor, to account for the pattern of the multicellular organism.

Beyond question the nuclear substance is of fundamental importance in relation to the specific hereditary constitution of the protoplasm of a particular species. I have tried to show, however, that this specific constitution represents, not organismic pattern, but the material or substratum on which this pattern is superimposed as a physiological order which merely determines what potentialities shall be realized and where each realization shall occur. There is no necessary conflict between this conception of organismic pattern and the theories of chromosomal pattern. Whether the chromosomes shall finally prove to be relatively simple fluid crystals, or linear series of definitely localized "factors," or something dif-

ferent from either of these, it remains true that, so far as organismic pattern is concerned, they represent merely potentialities. For the realization of different potentialities in different cells or cell groups, all of which originally possess all the potentialities, a pattern on a larger scale is necessary, and this pattern must be of external origin.

THE NATURE OF GROWTH[1]

DR. H. S. REED
UNIVERSITY OF CALIFORNIA

I

ONE of the most conspicuous characters of organic beings is their power to grow—to become what, at a previous stage of their existence, they were not. The acorn becomes an oak tree, the helpless babe becomes an Aristotle. What is the cause of the transformation? What forces are involved in these non-reversible processes which we term development, or growth? The oak tree can not be put back into the acorn, neither can the man be put back into the baby. It is needless to say that much of our philosophy of the universe has arisen from the attempt to answer questions which man has asked concerning these phenomena. In biology there have been two distinct schools—the vitalists, who held that the processes in organic beings are qualitatively different from those occurring in other forms of matter, and the mechanists, who hold that the differences, if any, are merely quantitative. The vitalistic naturalists have attempted to explain the organism in terms of a soul either connected with or separate from some superior soul. The mechanistic naturalists have attempted to explain the organism in terms of mass, time and space. It is not necessary to judge the successes or failures of the two schools in the present discussion. I do feel constrained, however, to remark that the students of the growth process (especially the growth of plants) have busied themselves with the irrelevant and unimportant aspects of the subject and have neglected most of the problems which might explain

[1] Read at a symposium on "Growth and permeability," held by the Pacific Division of the Plant Physiological Section of the Botanical Society of America, Los Angeles, September 19, 1923.

growth. In the words of a recent article by Kidd and West:[2]

> A few fundamental principles are necessary for the study of growth and development. These are conspicuous by their absence in existing text-books of plant physiology, which excel in the assemblage of interesting curiosities and of uncorrelated details. The phenomena of *normal growth* seem to call for further study and analysis and for the application of mathematical treatment.

In view of the great diversity of organic life and of its complex manifestations, it is not surprising that biology should be one of the last of the sciences to abandon the grab-sample methods of study with which man originally tackled the problems of the universe.

It is my purpose to show how the problem of growth may be quantitatively studied by the application of methods already in use in other departments of science and how their use simplifies the problem. I think it fair to assume that most of us have come to the realization that it is necessary to extend the application of quantitative methods in the study of physiology. In addition to measuring the factors of the environment, we must measure the response of the organism. Of the many responses of organisms, the two of paramount biological importance are growth and reproduction, yet their study has been long neglected.

II

One of the first results of the application of quantitative methods to biological processes is the discovery of their continuity. The organism of to-day is the organism of yesterday plus or minus a certain number of molecules of water, carbohydrate, *et cetera*. The processes of hydration and dehydration which play such an important part in the changes of carbohydrates, proteins and fats are mainly brought about by oxidases, reductases and other enzymes. These processes go slowly in comparison with many chemical reactions and are often reversed, but they are the principal centers of metabolic activity in the organism.

[2] *Ann. Appl. Biol., 6:* 2, 1919.

Studies of growth phenomena should eventually lead to a consideration of the energy relationships involved. We can not but believe, in the absence of contrary evidence, that the growth processes of living organisms are manifestations of the energy relationships prevailing in other parts of the universe. The study of these energy relationships ought not, therefore, to be disregarded. Our aspirations for such knowledge, which have received so much encouragement from the discovery of the principle of the conservation of energy, can never be entirely suppressed.

For purposes of this discussion the major phases of growth will be considered to be enlargement, differentiation and senescence. The organism which begins as a single cell becomes larger. As it becomes larger, its parts become differentiated. The little seedling is originally little more than a slender rod. But the rod grows and becomes differentiated into shoot and root, and the former undergoes further differentiation into vegetative and reproductive organs, each of which may become highly complex in structure and function. These processes constitute development or growth. Following them come changes which betoken the end of the growth process, leading to senility and eventually to death.

III

If we concur in the view that growth is an increase in size accompanied by a differentiation in members, we may inquire concerning the factors which are related thereto.

The organism acquires materials from its environment and by means of them becomes larger or different. Water is one of the substances which is acquired in large quantities and is of general importance for vital processes. A plant's ability to grow is, therefore, highly dependent on its ability to absorb water. The structure of most organisms is such that considerable water must pass through them every day. The lack of no other sub-

stance (oxygen excepted) is more quickly or keenly felt than the lack of water. These statements are so evident and so trite that you are perhaps impatient with me for taking the time to make them. My purpose in introducing them is to call attention to the dependence of growth upon imbibition. MacDougal[3] has unquestionably shown that imbibition is dependent upon the colloidal state of the cell and that the amount of water imbibed depends as much upon colloidal state as on access to an adequate supply. From MacDougal's experiments it is evident that proteins have the greatest water capacity in acid solutions and that pentosans show the greatest degree of hydration in neutral or slightly alkaline solutions. Miss Carey[4] has shown that gelatin absorbs more CO_2 after soaking in water and still more after soaking in HCl solution than the fresh gel absorbs. This may be due to the fact that gelatin absorbs more water from acid solutions than from pure water. Slight changes in the conditions cause conspicuous changes in the rate of imbibition. The ions of the common constituents of soil solutions have varying effects upon the imbibition and swelling of protoplasm and other colloidal substances. The studies on this question are legion, but they too often concentrate their attention on the water and ions which go through the cell colloids, whereas the importance belongs to what stays in them. In the case of root cells of a terrestrial plant the transmitted substances are obviously important because from them the rest of the plant acquires many of the necessary materials.

IV

The growth of an organism usually begins at a slow rate, gradually increases for a time, then becomes slower until it stops. The same is true of an autocatalytic reaction. It begins slowly, but as more of the catalyzing substance is produced, the reaction proceeds at an in-

[3] MacDougal, D. T., Carnegie Inst. Publ., 297, 1920.

[4] Carey, C. L., *Physiol. Res.*, 2: 407–432, 1923.

creasingly rapid rate. As the supply of reacting substances is used up, and as the products of the reaction increase in amount, the reaction slows down and comes eventually to a stop.

Robertson[5] has shown that the rate of a reaction like this may be expressed by the differential equation

$$\frac{dx}{dt} = kx(A - x)$$

When integrated this becomes

$$\log \frac{x}{A - x} = K(t - t_1)$$

In these equations x = size of the plant at time t; A = final size of the plant; t_1 = time at which half the final size is reached; k = a constant; and $K = Ak$.

This equation has been used to express the growth of sunflowers,[6] of pear shoots,[7] of cucumber leaves,[8] of lemon shoots,[9] and of white rats.[10] The production of flowers on the cotton plant,[11] of ammonia by ammonifying bacteria,[12] and of carbon dioxide by yeast[13] follow the same equation. The results of these and of other investigations have shown the general applicability of this equation to the study of the growth of organisms. Considering the nature of the materials studied, it must be said that the equations give results of satisfactory accuracy.

V

Our next question may well be: "What do these equations teach us concerning growth?" "Are we to regard

[5] Robertson, T. B., Arch. Entwicklungsmech., 25: 581–614, 1908.
[6] Reed, H. S., and Holland, R. H., *Proc. Nat. Acad. Sci.*, 5: 135–144, 1919.
[7] Reed, H. S., *Jour. Gen. Physiol.*, 2: 545–561, 1920.
[8] Gregory, F. G., *Annals of Bot.*, 35: 93–123, 1921.
[9] Reed, H. S., *Proc.* Nat. Acad. Sci., 7: 311–316, 1921.
[10] Reed, H. S., AMER. NAT., 55: 539–555, 1921.
[11] Prescott, J. A., *Annals of Bot.*, 36: 121–130, 1922.
[12] Miyabe, K., Soil Science, 2: 481–497, 1916.
[13] Rippel, A., Ber. deut. bot. Ges., 37: 169–175, 1919.

them as evidence of physiological principles, or merely as evidence of successful jugglery?" Some physiologists maintain that the resemblance between observed and calculated values is entirely superficial and without significance.

This is an important question, and no effort should be spared in attempting to answer it. It seems to me that we are at the beginning of a new epoch in plant physiology and that we have a responsibility in orienting our ideas to the wider view we are gaining.

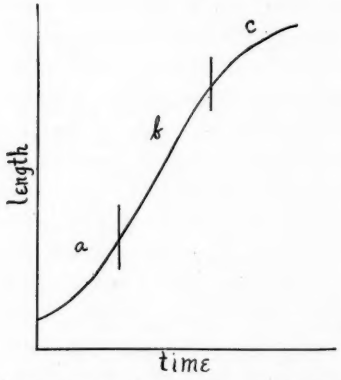

FIG. 1. The growth of pear shoots calculated from the equation

$$\log \frac{x}{114 - x} = .0242\,(t - 47.4)$$

The S-shaped curves (Fig. 1) representing the growth of an organism may be divided into three parts. The lower part (*a*) represents the initial period in which the size increases rather slowly; the middle portion (*b*) represents the period of most rapid growth; and the third portion (*c*) represents the period commonly known as the period of senescence.[14]

Since the curve plotted from the equation represents the growth of a wide range of organisms, we must guard against giving it too narrow an interpretation.

The growth represented by *a* undoubtedly is the result of an increase in the number of cells when they increase

[14] Priestley, J. H., and Pearsall, W. H., *Annals of Bot.*, 37: 239–249, 1922.

at a geometrical rate (the growth is an exponential function of time), while the supply of raw material in the environment is greatly in excess of the absorbing capacity of the organism. In the case of yeast and bacteria the number of cells is small in proportion to the volume of the nutrient medium. In the case of the higher plants, the young seedling or young shoot first draws on accumulated products of metabolism of the parent plant, gradually supplementing them with products synthesized in its own tissues. In the case of mammals, the young are utilizing during this period the nutrient fluids of the mother, which are especially rich in growth-promoting materials, and are gradually supplementing them with food from other sources.

The portion of the curve represented by *b* covers the period of most rapid growth and is the most conspicuous to the observer. Many physiologists who have studied growth have confined themselves to this part of the growth process. During this time the increase in size is almost (but not exactly) proportional to the time, *i.e.*, the cells increase (in size or number) at an arithmetical rate. We may assume that during this period the catalyst of growth is abundant, but that the growth is conditioned upon the rate at which the organism can obtain from the environment the materials upon which the catalyst can work. During this period growth is more apt to be influenced by fluctuations in the external medium than in the other two periods. In many cases growth during this period must depend upon the rate of oxygen absorption, and in the case of green plants, upon the rate of carbon-dioxide absorption. In all cases it is closely conditioned upon the rate of water absorption, and may therefore depend upon the rate at which acids are produced.

The last portion of the curve covers the period of senescence. The growth during this period comes to a point where no further increase in size takes place; as a matter of fact, some organisms may shrink because of the loss of water. The portion of the curve represented

by *c* is symmetrical with *a* in the case of most organisms whose growth curves have been studied. Both portions are obtained when the values of $\pm K(t - t_1)$ approach a maximum. During the period represented by *c* the cells increase in size or number at a rate only slightly greater than that at which cells die. Since the means for obtaining material are well developed, and the supplies of external material are not commonly depleted, we must assume that something has happened to retard the growth of the organism. In our present state of knowledge it seems most logical to assume that the catalyst which directs growth has become inactivated to such a degree that it no longer functions as formerly. Leaving aside the combined effects of limiting factors, there is still an inner factor which puts an end to further growth. We may plant a rose bush and an apple tree in the same garden and give each the best of conditions, yet the rose bush will never attain the size of the apple tree. Something in the internal organization of the rose bush terminates its growth at a certain point within a few years, whereas the apple tree will grow many years before its growth is terminated.

The study of growth of living organisms reveals an almost universal action of some sort of inhibiting substance which sooner or later brings growth to a stop. It has long been known that many micro-organisms produce substances which are inimical to prolonged growth. In many cases the stale culture medium may be reconditioned by boiling or by treatment with solid absorbing agents.

The condition known as dormancy in seeds, buds, bulbs and tubers must be also referred to the action of some substance (in some cases volatile esters[15]) which prevent growth until they have been eliminated or destroyed.

Certain internal secretions of the animal body are known to inhibit growth and to perform very definite form-regulating functions. The term *chalone,* proposed

[15] Mazé, P., Compt. rend. acad. sci. (Paris), 151: 1383–1386, 1910.

some years ago by Henry Fairfield Osborn,[16] is an appropriate and convenient designation for this class of substances.

The evidence for the existence of growth-inhibiting substances or chalones in the higher plants is accumulating. Loeb,[17] Appleman,[18] Halma[19] and the writer[20] have obtained evidence that the growth of plants in different families is regulated by chalones of some sort.

It is no part of my present purpose to discuss the nature of the evidence, neither will time permit a consideration of the criticisms advanced by those who find it difficult to abandon the timeworn, traditional viewpoint that growth regulation depends upon the presence of nutritive materials. My intention is to discuss the rôle of this class of inhibiting substances in the growth process. Since the old ideas upon this subject have never given an adequate explanation of the nature of growth, it may be well to indicate the bearing of these recent investigations upon the problem. The whole question is greatly simplified if we assume that the chalone is some sort of a substance which inactivates the growth-catalyst. Substances of this nature are well known in zymology and in physical chemistry. Iredale[21] has shown that the catalysis of hydrogen peroxide by colloidal platinum may be inhibited by gelatin, as well as by several other colloids. It is entirely unnecessary to assume that the chalone of every organism belongs to the same class of chemical substances. Colloids, acids, esters or even carbohydrates may act in different cases to retard the growth process of different organisms.

It is highly important to understand that the growth-retarding agencies are present from the beginning of the

[16] Osborn, H. F., "Origin and Evolution of Life," 1917.

[17] Loeb, J., *Science*, 46: 547, 1917.

[18] Appleman, C. O., *Bull.* 212, Md. Agr. Exp. Sta., 1918.

[19] Halma, F. F., unpublished results.

[20] Reed, H. S., and Halma, F. F., *Univ. Calif. Publ. in Agr. Science*, 4: 99–112, 1919; *Plant World*, 22: 239–247, 1919; *Jour. Agr. Res.*, 21: 849–876, 1921.

[21] Iredale, T., *Journ. Chem. Soc.* (London), 121: 1536–1542, 1922.

process. The amounts may be for some time so small that they have no material effect upon the catalyst, but, as time goes on, they accumulate and gradually inactivate the catalyst. Their retarding action may be increased or decreased by changes in the external medium whereby the absorption of oxygen or of other substances is altered, but the effect of external conditions on the form of the growth curve is not as great as generally assumed.

VI

The concept of growth-inhibiting substances throws much-needed light on the problem of cyclic or periodic growth. Many, if not most, organisms have more than one growth cycle. The intra-seasonal cycles in the growth of shoots of certain fruit trees are well marked.[22] Studies on the fluctuating growth of apricot shoots[23] produced evidence that their growth rate was alternately increased and diminished by some factor which varied harmonically during the growing season. So far as the evidence now in hand permits, it seems logical to assume that cyclic growth is related to the accumulation of some growth-inhibitor of a colloidal nature which inactivates the catalyst. After a time the physical or chemical state of the growth-inhibitor is altered, or the amount of catalyst present is in excess of the inhibiting power of the materials, and a new cycle of growth is begun.

VII

This discussion has dealt primarily with the equation for autocatalysis

$$\log \frac{x}{A-x} = K(t-t_1)$$

without any intent to ignore the other equations which have been used to express the growth of organisms. For some purposes Wilhelmy's equation,

$$x = A(1-e^{-kt})$$

[22] Reed, H. S., *Jour. Gen. Physiol.*, 2: 545–561, 1920.

[23] Reed, H. S., Natl. Acad. Sci. *Proc.*, 6: 397–410, 1920.

is well suited to express the growth curve. Blackman[24] has shown that for short intervals the growth of a plant may be expressed by the compound interest formula

$$W = W_0 e^{rt}$$

where W = dry weight of the plant at time t, W_0 = initial dry weight of the plant, r = the rate of interest, or "efficiency index" of dry-weight production, and e = the base of the natural logarithms. It is evident that $r = log_e W - \log_e W_0$.

Mitscherlich[25] proposed the formula

$$\log (\sqrt[n]{A} - \sqrt[n]{y}) = \log \sqrt[n]{A} - c.x$$

where n = a variable indicating the probable number of environmental factors, A = maximum possible dry weight, y = dry weight at time x, the time x being expressed in vegetative periods of arbitrary length.

It is not my present intention to discuss the relative merits of one or another of these equations but to stress the importance of expressing growth as an orderly dynamic change in which increase in size is equal to an exponential function of time. He who wishes may discard any or all of the equations mentioned, but there is no escape from the conclusion that the growth of plants should be studied as a problem in chemical mechanics.[26]

VIII

Without attempting to discuss additional data we may stop now to inquire what we may conclude from the facts already presented.

Primarily, it seems that growth is a dynamic process which causes enlargement and differentiation in organisms. It seems to me that these facts show a continuity in the growth process which has hitherto been unappreciated by physiologists. If we will replace our former ideas of the haphazard character of growth by the idea

[24] Blackman, V. H., *Annals of Bot.*, 33: 353–360, 1919.

[25] Mitscherlich, E. A., Landw. Jahrb., 53: 167–182, 1919.

[26] The reader interested in a critique of various equations should consult Schüepp, Ber. deuts. bot. Gesell., 38: 193–199, 1920, and Rippel, Jour. f. Landw., 70: 9–44, 1922.

that it is a slow chemical reaction of the first order, our ideas will more nearly accord with the observed facts. This may be nothing more than exchanging the idea that organic beings are the sport of the gods, for the idea that they are the result of a divine harmony achieved through the operation of natural laws.

The question which is often raised by those who have been accustomed to think of the organism in terms of its environment is, "How does the organism manage to maintain such an even growth rate in spite of the myriad fluctuations in its surroundings?" This applies especially to plants. Animals having the power of movement can, to some extent, get out of unfavorable surroundings, but the higher plant is fixed, and must take the environment as it finds it. We must admit that raising or lowering some essential factor, such as heat, to the death point or near the death point, will so alter the growth rate as to invalidate the foregoing assumptions. But, unless the conditions become too nearly lethal, organisms show a constancy in their development which merits our attention and study.

Our understanding of the behavior of the organism will be clarified if we apply the theorem of Le Chatelier, a theorem which has been derived from physico-chemical conceptions. It may be stated as follows: If a system in equilibrium is subjected to a constraint by which the equilibrium is shifted, a reaction takes place which opposes the constraint, *i.e.*, one by which its effect is partially annulled. In all cases, whenever changes in the external condition of a system in equilibrium are produced, processes also occur within the system which tend to counteract the effect of the external changes.

Let us see if we can apply this theorem. A plant growing in the field is in dynamic equilibrium with its surroundings, otherwise it is dead. If cloudy days or drought or other changes ensue, the activities of the plant take such a course that the effect of the changed conditions is minimized, and the growth rate generally suffers

little change. This appears to be an integration of the activities of the plant which results in its specific response—a response which is far more dependent upon the inherent nature of the plant than upon the surroundings.

I am aware that many, if not all, of the views here expressed will be criticized, and often adversely, in the next few years. If there be anything of truth in them, it will be sifted out. Undoubtedly I have erred in the direction of over-simplification, but this is due to an attempt to lay aside, so far as possible, all irrelevant matters and to discuss growth simply as a problem involving slow transformations of material at a rate proportional to time.

CONCERNING THE HOLLOW CURVE OF DISTRIBUTION

JOSEPH CONRAD CHAMBERLIN

STANFORD UNIVERSITY

In his recent book, "Age and Area," J. C. Willis advances a number of interesting hypotheses concerning the origin, growth and differentiation of species, genera and higher groups, and, particularly, the spread of species geographically. In the development of his theories he leans very heavily upon the deductions drawn from a statistical treatment of certain phases of systematic biology. The graphical plotting of nearly or quite all such data, so far as it involves frequency of occurrence as contrasted with size (in its most general sense), gives rise to the so-called hollow curve. It is this particular phase of his work to which I desire to call especial attention.

This paper is not presented as a criticism of any of Willis's theories but simply to call attention to some of the curious features presented upon a more or less complete analysis of this curve. The constant occurrence of this characteristic hollow curve primarily aroused my interest in this problem and my first motive was to try to determine to what extent, if any, it carried a meaning to the systematic biologist. If the curve does express some actual biological fact or facts in a useful form, it would be well worth while for it to be more generally understood and utilized. My own convictions as to its value are as yet somewhat unsettled. Certainly, it seems to bear considerable significance in certain cases, at least.

I am particularly indebted to Professor G. F. Ferris, of Stanford University, for valuable suggestions and criticisms as well as for aid in the gathering of most of the data presented herein. I am greatly indebted to Mr. Carl D. Duncan, of Stanford University, for reading the manuscript and for many helpful suggestions and criticisms.

As Bateson has already stated in his review of "Age and Area," there is some difficulty in following Willis in his arguments and conclusions, since nowhere does he give a clear unembellished statement of his hypotheses and the reasons for believing this particular curve to be of such profound importance as confirmatory evidence of them. This difficulty may be due more to the enveloping abundance of evidence than anything else. Consequently, as a preliminary to the results of my own investigations, I am stating his propositions and giving what I interpret to be the substance of his reasons for their support.

Briefly, the proposition in the first case is this. If we study the fauna or flora of any given geographical unit, we find in *all* cases that more genera are represented by but a single species than by two species; more with two than with three and so on. Then, if we plot the frequency with which the genera of various sizes appear against their absolute size (using, in this and other cases, the number of included species as a criterion), we obtain the hollow curve in its typical form (frequency to size).

Secondly, if we classify any group of plants or animals according to the geographical extent of their range (as determined by the most outlying stations), we similarly find more species occupying small or limited areas than medium or large areas. Plotting the data again gives us our hollow curve (frequency to area).

Thirdly, we find a more or less close correlation between the area over which a genus ranges and its size. Thus, it is found that genera of limited geographical distribution tend to be small, particularly monotypic to tritypic. Genera which are wide ranging, on the other hand, tend to be large, *i.e.*, to include many species. Taken on the average, the concordance is remarkably close. The curve obtained is very close to or identical with the usual hollow curve (area to size).

Fourthly and finally, if we take any given group of organisms without regard to faunal or floral restrictions, and plot the frequency with which genera of certain size

appear, we again obtain the hollow curve, monotypic genera being most numerous, ditypes next, then tritypes and finally a straggling group of larger genera (frequency to size). This proposition is essentially the same as number one.

As Willis emphasizes at every point, the mechanical regularity with which the hollow curve appears seems to call for some more or less mechanical explanation. With this in view he advances a number of hypotheses to explain certain phenomena here made apparent for the first time. He also and primarily utilizes it as support for his theory of age and area, which was first and principally based upon a study of the relationship and distribution of endemic floras, and a very good if not conclusive demonstration of the essential slowness of biotic geographical dispersal. In the following paragraphs, then, I shall give a bare statement of the most important of these hypotheses or explanations, but without any of the abundant supporting evidence given by Willis in his book.

The statement of his law of age and area will give us with essential completeness his ideas concerning the first two propositions outlined above. It is quoted verbatim from "Age and Area."

> The area occupied (as determined by its most outlying stations), at any given time, in any given country, by any group of allied species at least ten in number, depends chiefly, so long as conditions remain reasonably constant, upon the ages of that group in that country, but may be enormously modified by the presence of barriers, such as seas, rivers, mountains, changes of climate from one region to the next, or other ecological boundaries and the like, also by the action of man and by other causes.

To him the approximate correlation between the size of the genera and the extent of territory over which they range means that on the average species and genera of small or limited distribution are, phyletically speaking, young, while wide-ranging species and genera are on the average much older. 'The fact that so many of these "young" forms occupy such relatively minute areas as is shown by a study of the endemics of Ceylon and New

Zealand is advanced as "proof" that species arise by mutation as contrasted to the theory of evolution by infinitesimal variation, which theoretically requires a large area for specific and generic birth. Relic genera and species are believed to comprise but a small minority of the total number of forms of limited geographical distribution.

Another conclusion, and to me, so far as this study is concerned, the most interesting one, is that species and genera must arise more or less mechanically and regularly to give rise to such a curve. Likewise, the spread of species must on the whole approximate, in *related* groups, a more or less constant rate of dispersal.

To begin with we must remember, however, that the mere conformance of a curve derived from actual data to the curve postulated by a theory does not positively prove the thesis. It is in the nature of negative evidence. In other words, as we already know from a study of other curves, there may be more than one hypothesis whose theoretical curve will coincide with that derived from actual data.

The essential postulate that a species will occupy less territory when young than when considerably older is one which I think few will deny. It is not this, then, which is open to question, but whether the correlation between age and area is maintained with sufficient accuracy to enable the use of one known factor to deduce the other is decidedly more so. In other words, while admitting the theory of age and area as fundamentally true, can we believe that in the face of the innumerable environmental factors of a modifying nature, particularly barriers, that the correlation will be maintained to any recognizable extent? Willis maintains and ably defends this thesis, and this is largely the essence of his theory. The strongest point in his argument so far as this problem is concerned, then, is the close correlation obtained (as a whole and on the *average*) between the absolute size of genera and the area over which they range.

In any case a second hypothesis, as ably and logically defended, has not, as yet, been forthcoming and hence in any work I have done toward amplifying or modifying his conclusions, I am assuming for this purpose its fundamental soundness. In this connection I wish definitely to state that I am not committing myself *positively* to any of the conclusions arrived at. They are merely presented as more or less interesting possibilities or in some cases probabilities, with the hope that they may stimulate others to pursue this line of investigation further and determine more or less accurately their true value.

Willis's work was based almost exclusively upon plant studies. Mine are almost exclusively upon animals, and it is possible (although I think scarcely probable) that Willis would object to authorizing such a wholesale transference of his theories and conclusions. There is, however, so far as I have been able to discover, no difference between the two. It is possible that the age or size to area correlation would not hold so closely with animals as with plants, but even this appears doubtful to me in the light of my investigations.

While admitting the sound points in his arguments on the one hand, we must not overlook a considerable number of difficulties and criticisms which the theory must surmount on the other. Of these the first in my opinion is the *quality* of our systematic work. Is the quality and completeness of our systematic work sufficient for the drawing of any very far-reaching conclusions on the basis of the data which it supplies? For example, one of the most puzzling features about the whole problem was the appearance of apparently identical results from all types of work, and consequently from all types of data, good, bad and indifferent. That this similarity is not quite as great as it superficially appears is one of the points I hope to establish further on.

In this connection let us consider the Coccidae or scale insects in relation to Willis's fourth proposition, *i.e.*, frequency to generic size. Probably almost every worker

in this group will agree that the existing classification is but a very poor expression of the actual biological conditions within the group. It is quite safe to say that very few of the genera within this group as it stands at present are actual expressions of biological fact. It is of course true that in part genera are subjective creations and have, to a certain extent, no "actual" existence in nature and consequently there is apt to be wide divergence of opinion as to their limits. Yet in spite of this, there is doubtless considerable basis in actual biological fact for genera. This is not a place to enter far into a discussion of the Coccidae, but some illustrative examples may be given.

In Figure 2-B is plotted the curve as based upon the Fernald Catalogue of the Coccidae for the world. This

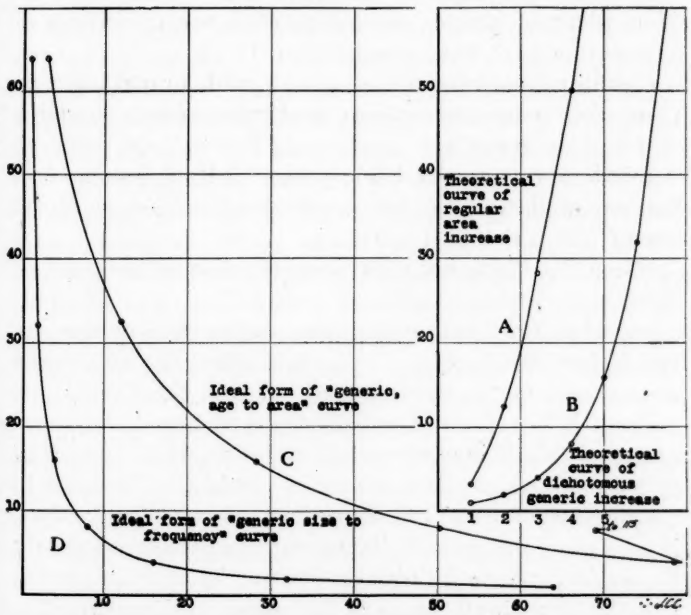
Theoretical curve of regular area increase
A
B
Theoretical curve of dichotomous generic increase
Ideal form of "generic, age to area" curve
C
Ideal form of "generic size to frequency" curve
D

FIG. 1, A–D. "Ideal" curves of generic-specific and area increase. In A and B, the ordinate represents increase, the abscissa time. In C and D the abscissa represents size or area; the ordinate frequency.

catalogue is now over twenty years old and was conservative for its day. Consequently, the curve as obtained is an extremely conservative one and is based upon sets of genera, very few of which are now regarded in anywhere near the same light as they were at the time the catalogue was published. An analysis carried through the whole group will show actually a very profound modification of the genera, and yet the hollow curves from the data obtained from such an analysis would be, apparently at least, no different.

Such a rearrangement has actually been made in part, by MacGillivray. He goes far to the other extreme, and in his book, "The Coccidae," over 100 new genera are named. From this volume the curve shown in Fig. 2-H was constructed. As may be easily seen, the superficial appearance of these two curves is striking, although the MacGillivray curve is decidedly unbalanced, particularly toward the monotypic genera.

Hence we are moved to inquire whether data drawn from such sources are of any particular value. It seems odd that we should get even similar results from two such discordant sources, and it appears as though some factors other than those of a purely biological nature are at work.

From this it was seen that some standard basis must be devised for the comparison of such curves. For this purpose, what I am calling the ideal curve was obtained in two independent ways. In the first place, the hyperbolic appearance of the curve suggested that the simple formula of $XY = K$ would satisfy the conditions. As a corollary to this it is obvious that the number of *species* in any given generic class should theoretically be equal to the number of *species* in any other generic class. Thus, if we have a group with 100 monotypic genera we should theoretically have 50 ditypic genera, 33 tritypic genera and finally a single genus of 100 species. Consequently, the ideal curve for any group under consideration may be obtained by simply totalling the number of species in-

cluded and extracting the square root of the sum. (This is the method by which all the ideal curves in the accompanying figures were obtained.) This root, then, is equal to the K of our formula and determines the limits of our curve.

The second derivation of the ideal curve was deduced from purely theoretical conditions with the following ideas as guides:

(1) Other things being equal, phyletic differentiation and spacial distribution will vary directly with time.

(2) The ideal curve for any type of phenomena may be obtained by divesting it of all modifying factors and carrying the progression to its logical mathematical conclusion.

Thus, in order to obtain the simplest possible expression for the evolution of genera (or species), we may assume an original genus which gives rise by mutation to a new genus for each regular time unit or interval, which rate is effective, also, as applied to the mutant or daughter genera. This is a simple case of geometrical progression, mathematically expressible by the formula, $a + (a+1)^1 + (a+1)^2 + (a+1)^3 + \cdots$, in this case "a" being equal to one. It is the type of increase displayed by a regularly dichotomously branching diagram or by the reproductive activities of a single-celled organism increasing by simple binary fission. In other words, we have simply expanded this type of reproduction to account for the origin of genera and species. This parabolic curve is shown in Figure 1-B.

Then, by assuming that species arise at the same rate and by the same method outlined above for the genera and carrying our progression for convenience' sake to the number 64 and plotting the sizes of the genera obtaining at this particular point we find that the eight values we obtain coincide exactly with points on the ideal form of the curve, as determined by using 64 as our constant or curve limit in our first method. This curve, with the points obtained by this hypothesis indicated, is shown in

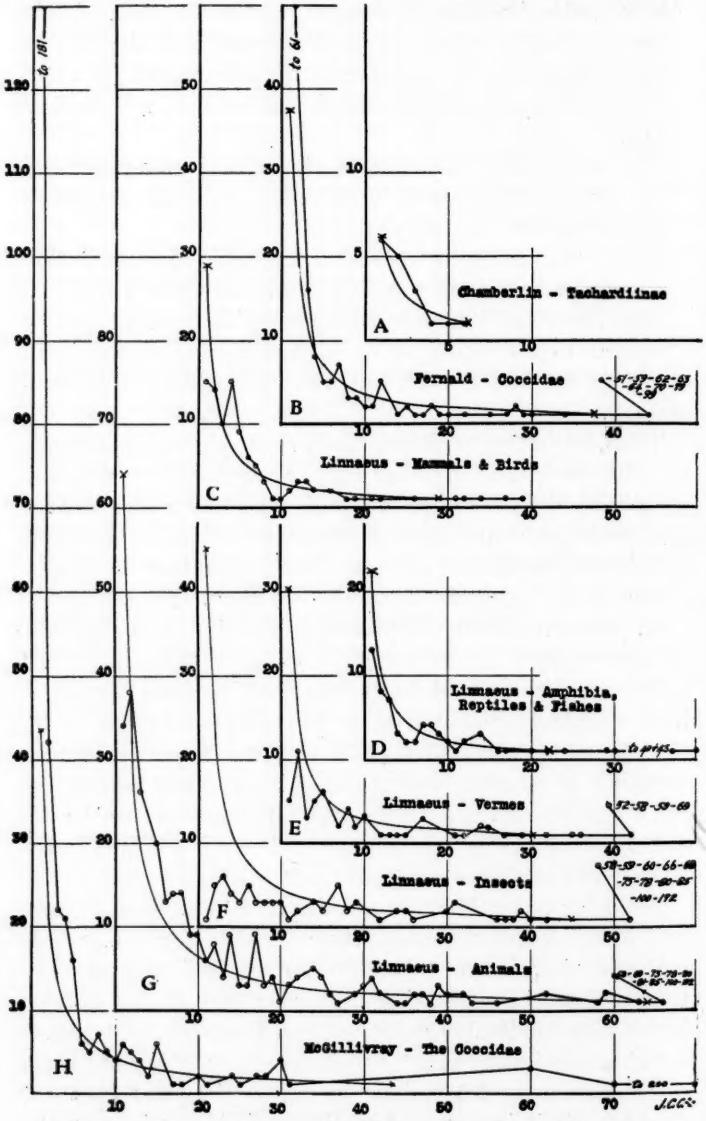

FIG. 2, A–H. Various hollow curves contrasted with their "ideals." In all cases the ordinate represents frequency; the abscissa, generic size.

Figure 1-D. This close correlation, then, seems to entirely justify the use of such a curve as a standard for our purpose.

To make clear this last method of obtaining the ideal curve we may carry out our progression as follows: 1—2—4—8—16—32—64. Then, assuming the same method and rate to hold for species, it is obvious that the original genus will now contain 64 species, there will be two genera with 32 each, 4 with 16, 8 with 8, 16 with 4, 32 with 2 and 64 with 1 species each. Thus it is clear that what we have here are simply the whole non-fractional frequency values on an $XY = K$ curve, with 64 as a curve limit.

In assuming our ideal curve it must be borne in mind that it is here advanced purely as a standard for rational comparison and *not* as a pattern.

To continue our comparison of the Fernald and MacGillivray curves, using our ideal curves as standards of comparison, we find that what seemed at first a strongly marked similarity is now no more than a more or less superficial resemblance, although even now sufficiently striking. The most obvious difference is in the enormous preponderance of monotypic genera in particular and small genera in general in the MacGillivray curve. It lies almost wholly above the ideal curve for such a number of species and there are nowhere near the "proper" proportion of larger genera. The Fernald curve very closely approximates its ideal, the only radical departure being in the slightly excessive number of monotypic and ditypic genera.

Now what seems to be the only reasonable conclusion we can draw from our study so far is this. While both these classifications are actually and seriously far from the truth, they are nevertheless of sufficient accuracy to show the underlying evolutionary tendencies of the group. On the other hand, they certainly can not be relied upon for detailed analyses.

For example, if we could assume that MacGillivray's data were 95 per cent. accurate, instead of (as a pure

guess) 75 per cent. or probably even less, it would be possible more or less safely to interpret the enormous preponderance of small genera, together with a paucity of large ones, as indicating that we had here a very rapidly evolving group and one which is comparatively young or else one that is probably senescent. On the same basis Fernald's curve would indicate a comparatively stable group—possibly near the maximum of the group's development.

Before going any further it might be well to note some other factors which also operate to give us the hollow curve. As we have already demonstrated, the theory of "mechanically regular" origin of species and genera will alone account for the curve. On the other hand, there is no reason for supposing that there are no other factors which could give the same result. In other words our curve may be, not the resultant of a single factor, but the combined effect of several. Willis discounts the possibility of relics or remnants of dying genera influencing his results to any extent. As before mentioned, he believes that, as compared to the enormous mass of newly evolving forms, relics will simply form a very small minority of the total. On the other hand, if we take a large group of organisms which has evolved to a racial maximum and final extinction, it is certainly beyond question that every species evolved in that group must have ultimately become extinct in one way or another, and consequently at some time or other must have posed as relics. In other words, the two are equalities. This objection to Willis's theory, however, is not as serious as it might at first sight seem. Thus, while there is no doubt that once a group has reached an evolutionary maximum and *racial* extinction has set in, we should expect (were all differential evolution brought to a standstill), that purely as a matter of chance a random killing out would maintain the curve unchanged to the final phase of extinction. On the other hand, such random killing would undoubtedly not occur and certain groups would become

extinct far earlier than other hardier ones. Nor can we assume a cessation of divergent evolution in a senescent group. We know as a matter of actual fact that right up to the final phase of their extinction, the ammonites and trilobites as well as other now extinct groups produced new genera, and as a matter of fact were probably evolving new generic types with as great if not greater rapidity than at any other time in their history. (Using, of course, as criteria, the same type of morphological characters for limiting genera arising in this final period before the extinction of the group that were applied to the genera existing before this "running wild" of generic and specific characters, as a final phase of their senescence had appeared. This proviso is necessary to obviate possible irregularities introduced by a sudden shifting of our generic concept.) Then the inevitable dying out of genera—either through actual extinction of included species or their transformation into new generic types (whether or no this latter occurs is questionable, the former not so) would tend inevitably to produce an increasingly greater and greater proportion of monotypes. Thus, large genera as a class would inevitably disappear first, *i.e.*, be reduced to smaller and smaller genera through the dying out of some of their members and metamorphosis of others, so that up to the final phase of their extinction we should have our curve not only maintained but accentuated. And as may be seen by a consideration of the above the preponderance of monotypes would or at least could be "new" beginners. From a study of our curve alone there would be some difficulty in determining whether or no we were here dealing with an adolescent or senescent group.

On the basis of our ideal curve, if we plot the *percentages* of monotypes we find them varying inversely with the size of the group under consideration. Thus the ideal curve for a group of one included species would show 100 per cent. monotypes; of four species, 50 per cent. monotypes; of nine species, 33 1/3 per cent. monotypes; of

16 species, 25 per cent. monotypes, and so on. In this connection the significant thing to observe is that these are all points on an ideal curve with 100 (per cent.) as a curve limit. Thus, it is obvious that the larger groups will have relatively the smallest proportion of their species included in monotypic genera.

It is interesting and significant in this connection to note that the monotypes plotted as percentages, against time, will, as evolution proceeds from birth to senescence and death, inevitably result in a double or descending and ascending hollow curve. Thus, if we assume a group arising monophyletically from a single genus and species, we have originally 100 per cent. of monotypes. This percentage will decrease in a descending geometrical series as differentiation and phyletic increase becomes effective, and the minimum percentage of monotypes will be attained at the maximum development of the group as a whole. Then, as the process of extinction begins, the percentage of monotypes will increase until, as the final phase of extinction is reached, we will have a single genus and species remaining or 100 per cent. of monotypes.

In brief, the minimum *percentage* of monotypes in any given group of organisms will be attained at the point of its evolutionary maximum of abundance.

Thus, if our period of observation of any given group could be extended over a sufficient lapse of time to determine in which way the general trend of monotypic percentage varied, we could say for certain whether or no we were here dealing with a declining or ascending group.

Then, with some other considerations in mind, such as ecological adaptability and geographic range, we could from a knowledge of the percentage of monotypes make some logical estimate of how far, in a relative time sense, our group is from either a maximum of phyletic development or from death.

If these deductions are true, they will enable us to understand why groups appear to arise with such sudden-

ness as is witnessed by the paleontological record and why they as quickly disappear, though they may have in between these two phases an enormously long period of comparative group stability.

From the above considerations it is seen that the relative percentage of monotypes possessed by any given group is in effect an index to the phylogenetic development of that group.

In consequence of the above considerations, it may be easily seen that our data must be of a considerably more accurate type than is the case with those given by either MacGillivray or Fernald. The peculiarities of these particular curves may be purely fortuitous.

As a third factor, tending, in practice, to produce a preponderance of monotypes, may be cited the methods of our systematic work. Comparatively few genera are given birth to, systematically speaking, with their full complement of species. In other words, our systematic knowledge is in large part very incomplete, so that many genera, as we now know them, will in the future undergo profound modification through the addition of newly discovered species and redefinition of generic limits. Large genera, as soon as they become unwieldy, are subdivided or split, a tendency certainly present and one which has been much deplored by some.

Is not this, however, exactly what actually occurs in nature? For example, we may be sure that certain characters, all of potential variability, possessed in common by an original group of species, will tend less and less to parallel each other's development as the group grows. This is, of course, a direct consequence of the specialization of these original characters plus their individual and independent "acquisition" of new features. In other words, we have our old group breaking down into new ones, or more accurately attaining a super-group rank. For example, two species may well possess 50 significant characters in common, but the chance of such a group now grown to include 50 species, possessing this same

series of parallelling characters, is very small. This "breaking down of genera" probably occurs in an inverse geometrical ratio to the size of the genera, being greatest in the larger ones. This is, of course, merely another expression of our previously stated theories of generic growth and differentiation.

To recur to our discussion. While the method of our systematic work will no doubt tend to produce a preponderance of monotypic genera to begin with, we may assume on the other hand that after a certain preliminary stage has been reached our data will assume the nature of a random sample and hence this factor will become negligible or non-existent. In this connection it is probable that comparatively few groups, among the animals particularly, have reached this stage. This is especially true among the insects, where we find that to date the majority of our species are known from very few records.

As a matter of fact there is no doubt that more species in our entomological literature are known from one record than from two, more from two than from three, and so on. In other words, we obtain our typical hollow curve, which in this case undoubtedly is a graphical representation of our systematic knowledge of the group and not of its phylogeny at all.

To get back to the main thread of our study. In order to determine just where we begin to get significant indications of our hollow curve, an analysis of the Systema Naturae of Linnaeus for the animals was undertaken.

The curve for the work as a whole was first plotted (Fig. 2-G). Then, to complete the study, the book was divided into four sections, each of which was considered separately. In the first of these curves, that for the birds and mammals (Fig. 2-C) (which group we may safely assume Linnaeus understood better than any other), we get a very good approximation to the ideal curve for the group, except for the fact that monotypes to quatrotypes are present in almost equal numbers. In the second curve, that for the reptiles, fish and amphibia (Fig. 2-D)

we have an even better correlation, since here we actually obtain a preponderance of monotypes, although the curve as a whole does lie below its ideal. The curve for the vermes (Fig. 2-E) and for the insects (Fig. 2-F), the other two classes considered, show scarcely a trace of the underlying "hollow" nature of the curve. We know of course that Linnaeus's actual classification of the vermes and the insects was undoubtedly more artificial than for the mammals and birds. The most surprising thing is the comparative "excellence" of the fish-reptile curve. Linnaeus surely had no more real comprehension of them than he had of the mammals and birds, and it seems probable that the approximation here obtained is due largely to chance, and possibly to the relatively greater incompleteness of his knowledge. The almost total obliteration of the curve for the vermes and insects is almost surely entirely due to his *real* lack of appreciation of their actual relationships.

Knowing the actually crude nature of his work (whatever its relative quality or value) it is surprising to note the fairly close resemblance of the Linnaean curves as a whole to the typical hollow curve form. However, as we may easily see, when compared to their ideal forms, the resemblances are largely superficial. Thus, all these curves (excepting the anomalous fish-reptile curve) are very irregular, they lie almost wholly below their ideals and the number of ditypic genera is greater than the monotypic ones. An obvious interpretation is that here we are dealing with incomplete and highly inaccurate data.

Then to determine the type of curve given by systematic work of the very highest quality, an analysis of Miller's Catalogue of the Mammals of Western Europe was made. Three curves were derived from the data given in this work. One of these recognizes subspecies (Fig. 3-A), one species only (Fig. 3-B), while the third and last is a curve in which the genera represented in Europe were plotted against their total (instead of just

the European) number of species contained in them. This latter curve is based upon Miller's own estimates as given in his catalogue, supplemented in small part by Trouessart's catalogue of the Mammalia. This latter curve is shown in Figure 3-C. A very interesting result is manifested. The curves based upon the species and subspecies, relatively, are essentially the same, the only noticeable difference being a small reduction in the number of small genera in the subspecific curve. The Miller and Trouessart curve, however, exhibits a very striking modification in that there is an almost total obliteration of the "hollow" in the curve. The monotypes (and some of the other smaller genera) obviously represent, in this case, the truly endemic or new genera of Europe, and as we see they are actually very few. The "humps" at six and twelve are misleading. They are primarily due to Miller's method of estimating the number of included species as "a half dozen" or "about a dozen."

This flattening of the curve is most striking and might indicate several things. The most obvious meaning would appear to be that we have here a territory which is comparatively new to invasion, or else one that is having its fauna rapidly exterminated, probably the former. The "newness" is demonstrated by the actually small number of precinctive genera and by the comparatively large numbers of genera here represented by one or two species, which in adjacent regions reach an extraordinary degree of abundance. Thus the region would appear to have something in the nature of a recent "overflow" fauna, obviously resulting in a preponderance of apparently small genera when the European species alone are considered. Then in addition to the above effect we have our curve somewhat accentuated by a number of undoubtedly dying genera.

It appears that this Miller-Trouessart effect should be of considerable practical value in the balancing and limiting of faunal and floral areas and life zones, inasmuch as

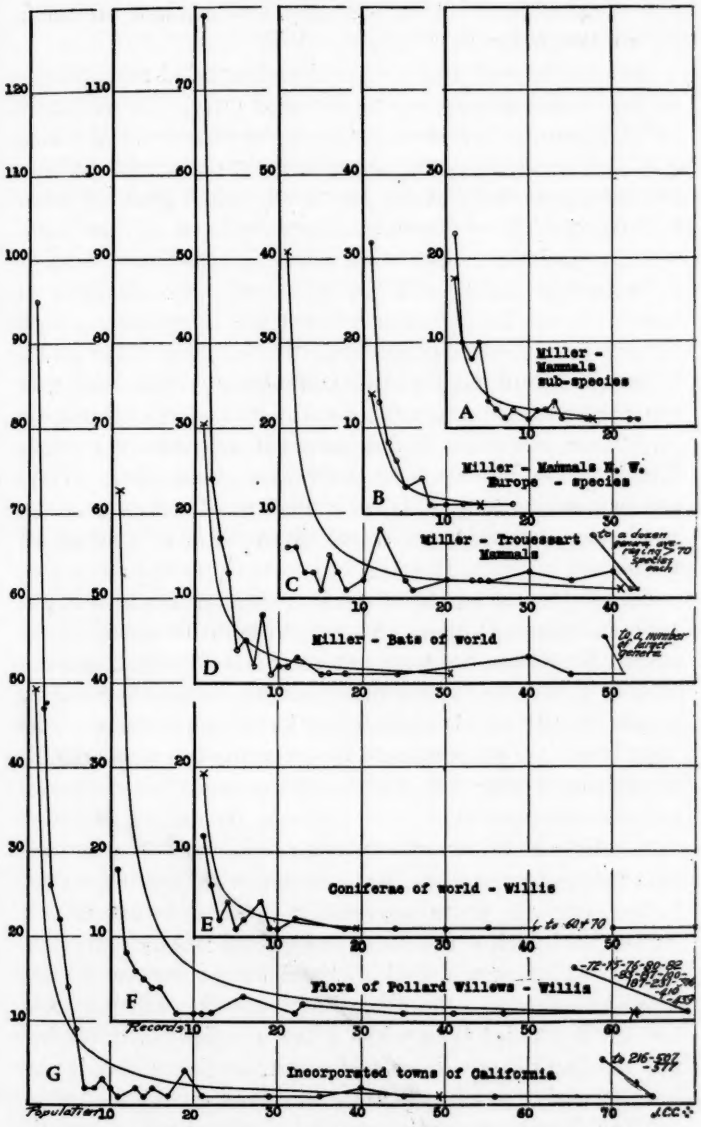

FIG. 3, A–G. More hollow curves and their "ideals." Unless otherwise stated the ordinates represent frequencies; the abscissa generic sizes.

the curve gives us a simple graphical method of weighing our data.

As may be seen at a glance the superficial resemblance of the Miller curves to the Fernald curve, for example, or (neglecting the ideal comparison curve) to the Linnaean fish-reptile curve is sufficiently close to enable us to state positively that only in the most general sense will the quality of the data affect the form of the curve, whether given alone, or contrasted with its ideal form.

In further substantiation of the above statement we may note the form given by plotting the genera of the bats of the world as given by Miller in his treatise on the "Families and Genera of the Bats." This work is scarcely as adequate as his catalogue of the European Mammals, inasmuch as his material was scantier, but in spite of this it is work of the very highest quality. We obtain a curve (Fig. 3-D) of almost as aberrant a nature as the curve based upon MacGillivray's "The Coccidae," which work stands in striking contrast, so far as quality is concerned, to that of Miller. Assuming the validity of Miller's data, the most obvious explanation of the great preponderance of monotypes as coupled with the numbers of larger genera is that here we are dealing with a relatively little known group. This is in accordance with what we know from other sources to be the case. Miller himself estimates that perhaps 60 per cent. of the group is known at present.

Finally, in this connection, are we safe in assuming that complete and accurate systematic data will give this hollow curve? I am personally convinced that it will. But should such a result be recognized in our nomenclature? At present I feel inclined to say no, with some qualifications. On the other hand, I feel much less positive on this point than when I first considered it. While recognizing the smallness of the numbers involved, it may be instructive to consider the following example.

Thus, having recently completed a monograph of the Tachardiinae or lac insects, I was interested in determin-

ing the form of the curve given by plotting the data here derived. There were 44 species recognized, which, on the basis of our theoretical curve, should give us six or seven monotypes, three or four ditypes and finally a single genus of six or seven species. As a matter of fact I had recognized nomenclatorially only four genera and two subgenera, which plotted give a perfectly straight line. On the other hand, I recognized and segregated groups and subgroups which, if considered as genera and plotted as usual, give a curve which practically coincides with its ideal (Fig. 2-A). Now then the question is this. Was I right or wrong in not recognizing all these distinct groups and subgroups as valid genera and should I have recognized them nomenclatorially? Certainly the characters used in separating them are no less constant and definite than those used by some systematists in distinguishing their genera. In other words, does the straight line lying wholly beneath its ideal curve given by my *named* genera and subgenera indicate an ultra-conservative classification, *nomenclatorially* speaking? At present I am not willing definitely to commit myself one way or another, but I should certainly be somewhat hesitant about proposing a series of new names for the groups and subgroups involved in this discussion, a procedure which without doubt would be followed by some workers. And which of these procedures is logically correct? It seems to me that if we may ever hope to attain a very close approximation to a natural system of classification we will have to devise some means for handling and distinguishing groups and sub- and supergroups whether or not we recognize them by names. The preceding discussion shows plainly that our hollow curve gives us an entirely new standpoint from which to rationally consider the "genus."

As an illustrative example purely, there is given the curve of the Coniferae (Fig. 3-E) as obtained by plotting the data given by Willis in his "Dictionary of the Flowering Plants and Ferns." It shows very beautifully the wide separation of the curve from its ideal and again

serves to emphasize the necessity of a standard of comparison such as our ideal curve, for a real appreciation of the peculiarities of individual curves. The curious irregularities in this curve seem to primarily call for a reinvestigation of the data involved. Assuming its validity, a point upon which I can voice no opinion, it seems to indicate that some very complex evolutionary factors have been at work modifying the generic ratios. Certainly more than senescence or phyletic differentiation is at work.

As a final interesting case, and one which opens up a number of interesting possibilities, is the curve for the flora of the Pollard Willow trees near Cambridge, England, as determined and reported upon by Willis and Yule. In this case species are plotted against the frequency of their occurrence in these willow trees. The willow tops, being in fact a virgin territory, may be considered as a new area being populated by an overflow of species from surrounding regions. Of course this is not strictly true, inasmuch as each willow top probably derived its flora independently from the surrounding territory instead of from the adjacent willows. However, as the curve shows (Fig. 3-F) we have here an ideal illustration for showing definitely that an overflow flora will independently give the hollow curve so far as age and area is concerned. As Willis stresses, a lucky accident may have placed a plant with a very poor dispersal apparatus among the first arrivals in these willows, but on the whole there is little doubt that the poorer the dispersal apparatus, the later on the average would the plant concerned gain a foothold in these places. And there is scarcely room for doubt that if the whole history were known, the species with the fewest records would represent the latest arrivals. The curve does indeed lie considerably below the ideal, but nevertheless it is remarkably similar in general form to typical examples.

I have not considered the correlation between age and area so closely, but what study I have given it clearly in-

dicates that the curve derived by plotting generic size to area lies definitely higher and beyond the corresponding size to frequency curve. This may be demonstrated theoretically by calculating its ideal form as was previously done for the generic size to frequency curve. It also works out very clearly in practice, but this data I am reserving for a future treatment.

Thus we assume the same type of generic and specific increase to hold as before outlined and in addition assume that species will at their inception occupy a circular area, whose radius may for simplicity be taken as equal to unity. Then assume that during each phylogenetic time interval, our species will spread radially a distance equal to unity. This gives our ordinary dichotomous progression modified by the "pi r squared" factor involved in determining the area of our theoretically circular range. As is shown in Fig. 1-A this is a considerably more rapid rate of increase than the ordinary dichotomous progression. Then, calculating our results at any finite point, here for the sake of convenience again taken at 64, we obtain the data graphically expressed in the curve shown in Fig. 1-C. As may be seen at a glance it lies entirely distinct from and beyond the corresponding curve for the phylogeny of our hypothetical group. In short, the rate of distribution in space (geographical distribution) is distinctly greater than in time (paleontological distribution).

As before mentioned, actual data, in many cases at least, conform very closely to the above derived hypothetical curve. In dealing with actual data it is of course obvious that extremely few cases of even approximately circular ranges will appear. This deficiency may in large part be obviated, especially in comparatively young groups, by taking the outlying stations of a given genus or species and calculating therefrom a theoretical center of dispersal and theoretical radius. This is what has been done in obtaining the results here referred to.

Before concluding it might be worth while to call atten-

tion to a large class of phenomena which give curves of striking similitude to the true hollow curve. Thus data giving an extremely skewed frequency distribution will fall into this class. For example, the wealth curve shows this resemblance perfectly. To illustrate this point there is given the curve derived by plotting the frequency with which incorporated cities of certain sizes appear in the state of California. Fig. 3-G. As here shown, the comparatively few incorporated towns of less than 1,000 inhabitants are lumped with the thousand class and as a result we obtain a beautiful and typical hollow curve. Actually we should have plotted these cities in their proper position and in that case the peak of the curve would have been slightly lowered and the curve drawn sharply downward to the base line at about the 300 population, size limit—giving us a splendid example of a sharply skewed curve.

It may be argued, then, that all these so-called hollow curves are nothing more than extreme skew forms. This position is not believed to be tenable, however, inasmuch as the nature of the data involved does not permit us to consider sizes nor frequencies of less than unity. A fractional genus or species has no valid existence.

To recur to one of the most interesting problems involved in this study, that is, as to whether we "should" obtain the hollow curve, granting for the moment at least the soundness of the theory of regular dichotomous generic increase, in view of the innumerable disturbing ecological factors. Personally, I believe it should. As a matter of fact, there is required just some such basic theory of as fundamental a nature as this to furnish an adequate reason for the curves' actual occurrence. And finally, while freely admitting the lack of positive proof for this underlying, theoretically ideal method of growth, nevertheless, the substantiation offered by the curve itself, as well as by the fact that we find this simple type of increase common to organic reproduction in general (may it not be a direct consequence of organic reproduc-

tion in general?), makes its use seem perfectly logical and safe as a working hypothesis. Its very simplicity is an argument in its favor. Naturally, we can not expect it to work out with the fluency of a mathematical progression, but at any rate this should not vitally impair its usefulness in practice. Modifying and "upsetting" factors are no more than the "impurities in our reaction." They correspond exactly to the contaminations and counter-reactions to be found in chemical combinations as they normally occur in nature.

In conclusion I should like to call attention to the vital need of more complete and accurate systematic work. What we need first of all, before we can proceed with any assurance, to prove or disprove such theories as have been advanced in this paper, is adequate and accurate data. It is no more than axiomatic to reiterate that results can be no better than the data upon which they are based (and they may be much worse), and it is only too obvious that much of the enormous mass of systematic work already done, does *not* conform to these requirements.

As in all other branches of science, accurate observation and data gathering must precede the formation of accurate generalities. A Kepler must precede a Newton. And here where we have complicating factors assuming such a tremendous rôle when coupled with the complexity of biotic phenomena in general, the need is obviously even greater. It is not too much to say that the gathering of accurate systematic data calls not only for the very highest type of individual observational ability, but also for the highest form of collaborative and correlative work. And in referring to systematic data, I mean it in its broadest sense. The careful and critical collection and organization of every scrap of evidence, from whatever the source, having a possible bearing on the phyletic affiliations of a group of organisms, constitutes in my mind true systematic work. And correlated with, and a vitally necessary part of this work, is the "bookkeeping"

or taxonomic nomenclatural handling of the group. After all, it is as necessary that the biologist be able to deal with species of known characteristics, as it is that the chemist be able to deal with chemically pure substances, and the name of an organism, insofar as it is accurately determined by a competent man, is the "trademark and guarantee" of our material. This point is only brought up here because it is only too apparent that many biologists, if not openly contemptuous of the taxonomist or, as I prefer to call him, the systematist, seem to forget that *his* is the tie that binds, in large part at least, whatever information their own researches have yielded into the general fabric of our biological knowledge.

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SHORTER ARTICLES AND DISCUSSION

A KEY TO THE EGGS OF THE SALIENTIA EAST OF THE MISSISSIPPI RIVER[1]

For the last two years the authors have been intensively studying the life-histories of the Salientia (frogs, toads, tree-frogs, etc.) of the Okefinokee Swamp, Georgia. A summary of one phase of the subject, namely, eggs, is herewith presented. Incorporated with this consideration of our southeastern species is a previous key to the eggs of our northeastern forms. The combination may be termed "A key to the eggs of the Salientia east of the Mississippi River."

Workers will discover the following species missing from the key because the authors themselves have had no opportunity to secure both fresh and preserved materials of these species:

Hyla andersonii—Eggs first found by T. Barbour and later by G. K. and R. C. Noble.

Hyla evittata—Eggs not known. Doubtless like *Hyla cinerea.*

Pseudacris ornata—Eggs not known.

Pseudacris nigrita—Eggs most certainly like *Pseudacris triseriata, P. feriarum,* and *P. septentrionalis.*

Rana areolata—Eggs not known, probably of *R. pipiens* type.

Rana cantabrigensis—Eggs very similar to *R. sylvatica.*

Rana septentrionalis—Eggs described by Garnier. After this paper went to press the authors and Mr. S. C. Bishop discovered eggs of this species. The description is: Egg mass submerged, a plinth; 1.5 × 3 inches to 3 × 6 inches; vitellus black or brownish above, white or yellowish below; vitellus, average 1.4 mm; range 1.3–1.6 mm; inner envelope, average 2.7 mm; range 2.4–3 mm; outer envelope, average 6.3 mm; range 5.6–6.6 mm. Season, June 25–July 25.

The season of breeding for species in the north is marked both at the beginning and at the end. Each species occupies four or five weeks except *Bufo americanus* and *Rana clamitans.* The exceptions may require two or three months for ovulation. In

[1] The investigation upon which this article is based was supported by a grant from the Heckscher Foundation for the Advancement of Research, established at Cornell University by August Heckscher.

In the summer of 1921 Mr. Francis Harper assisted in the field work of this study and in the summer of 1922 both he and Mr. Miles D. Pirnie gave valuable assistance to the authors in this same series of studies.

the southeastern states when once a species has begun, its season of breeding may extend throughout the summer or even into the early fall, dependent upon the high crests of precipitation. These species, although of a swampy region, wait for the rains

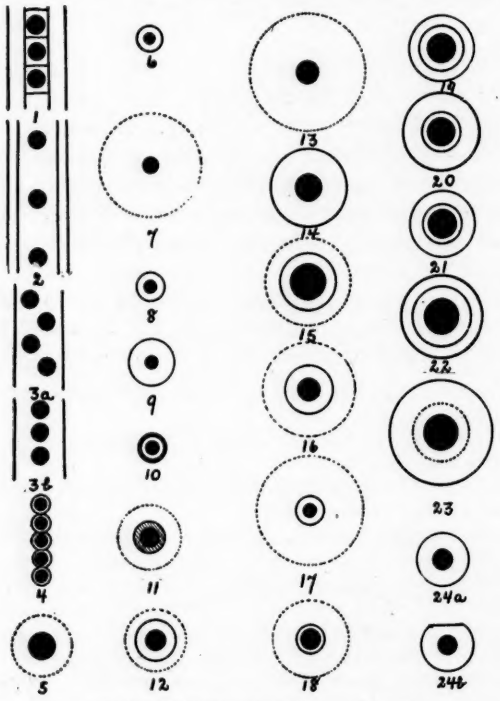

PLATE I. INDIVIDUAL EGGS. × 2.3

Fig. 1. Bufo americanus.
2. Bufo terrestris.
3. Bufo fowleri.
4. Bufo quercicus.
5. Scaphiopus holbrookii.
6. Pseudacris ocularis.
7. Pseudacris triseriata. (From Buffalo, N. Y.).
8. Hyla crucifer.
9. Acris gryllus.
10. Hyla squirella.
11. Hyla gratiosa.
12. Hyla cinerea.
13. Rana catesbeiana.
14. Rana virgatipes.
15. Rana grylio.
16. Rana clamitans.
17. Hyla femoralis.
18. Hyla versicolor.
19. Rana palustris.
20. Rana pipiens.
21. Rana sphenocephala.
22. Rana aesopus.
23. Rana sylvatica.
24. Gastrophryne carolinensis.

In Fig. 15 the vitellus was inadvertently drawn too large.

and in this reliance on precipitation they suggest our desert species of Texas and Arizona. Those which do not begin until June have at least eight to ten weeks of ovulation. This minimum period for a species of the south is the maximum period for a northern form. Species such as *Rana sphenocephala, Bufo terrestris* and *Acris gryllus,* which begin early in the season, breed during 25–30 weeks of the year, if not longer or from February to September or October.

The number of eggs in a complement may vary from 100 in the smallest species, *Pseudacris ocularis,* to 20,000 in *Rana catesbeiana,* the largest form. The range in the tree-frogs (*Hylidae*) is from 100 (*Pseudacris ocularis*) to 2,084 (*Hyla gratiosa*); in the toads (*Bufonidae*) from 610 (*Bufo quercicus*) to 8,000 (*Bufo americanus*); in the frogs (*Ranidae*) from 349 (*Rana virgatipes*) to 20,000 (*Rana catesbeiana*). The complements of the narrow-mouthed frog (*Gastrophryne carolinensis*) and the spadefoot (*Scaphiophus holbrookii*) are, respectively, 869 and 2,332.

The eggs of seven species, *Hyla cinerea, H. femoralis, H. versicolor, Rana catesbeiana, R. clamitans, R. grylio* and *Gastrophryne carolinensis* float on the surface of the water; the eggs of the other 17 species are submerged. In northern or southeastern states no form with buoyant eggs lays before May 10. The 11 or 12 early breeders have submerged eggs. These are usually with firm jelly envelopes except for *Pseudacris triseriata* and *Scaphiopus holbrookii,* which have the consistency intermediate between the firm jellies of early breeders and the loose surface films of late breeders. One form, *Gastrophryne carolinensis,* although it lays at the surface, has the most beautifully distinct, firm eggs of all the species considered.

The eggs were laid in camp and in the laboratory by mated pairs caught in the field. Later the eggs in the field were determined by these original checks. Occasionally the process of egg laying was observed in the field. Two species, one of the north and one of the south, were identified by the positive elimination of all the other resident forms.

The measurements and color descriptions are based on fresh eggs. Later, these were checked with preserved material. The eggs with loose outer envelope have the outer margin indicated by dots. In one species the vitelline membrane is far separated from the vitellus and the space is indicated by cross hatching. A summary of the egg characters of each species follows in the accompanying key:

A. Eggs deposited singly.
  a. Envelopes two.
    Outer envelope diameter 1.4 to 2.0 mm; inner envelope diameter 1.2 to 1.6 mm; vitellus diameter 0.8 to 1.0 mm; eggs brown above and cream below. Egg-complement, 942. Season, June 10 to August 21 (Fig 10) ..... *Hyla squirella*
  aa. Envelope single.
    b. Envelope 2.3 mm or more.
      c. Vitelline membrane far from vitellus, appearing as inner envelope 1.6 to 2.0 mm; outer envelope loose, glutinous, indefinite in outline 2.3 to 5 mm; vitellus 1.0 to 1.8 mm. Egg-complement, 2084. Season, June 10 to August 21 (Fig. 11) ..... *Hyla gratiosa*
      cc. No inner envelope or appearance as such, envelope firm, definite in outline 2.4 to 3.6 mm; vitellus 0.9 to 1.0 mm. Egg-complement, 241. Season April 15 or earlier to September 1 (Fig. 9) ..... *Acris gryllus*
    bb. Envelope 1.2 to 2.0 mm.
      c. Vitellus 0.6 to 0.8 mm. Egg-complement, 100. Season, May 16 to August 21 (Fig. 6) ..... *Pseudacris ocularis*
      cc. Vitellus 0.9 to 1.1 mm. Egg-complement, 800 to 1,000. Season, March 30 to May 15 (Fig. 8) ..... *Hyla crucifer*

AA. Eggs deposited in a mass.
  a. Egg mass, a surface film.
    b. Egg envelope outline always distinct, never lost in the mass; eggs firm and distinct like glass marbles, making a fine mosaic; envelope a truncated sphere, the flat surface above; envelope single 2.8 to 4.0 mm; vitellus 1.0 to 1.2 mm; color black above and white below. Egg-complement, 869. Season, May 21 to August 17 (Fig. 24) ..... *Gastrophryne carolinensis*
    bb. Egg envelope outline indistinct, more or less merged in the jelly mass; jelly glutinous; egg brown above, cream or yellow below.
      c. Egg packets small, masses seldom if ever over 20 sq. in. (125 square centimeters), or 4 by 5 inches in diameter (10 by 12.5 cm).
        d. Inner envelope large 2.2 to 3.4 mm; outer envelope 3.2 to 5.0 mm; vitellus 0.8 to 1.6 mm. Egg-complement, 343 to 500. Season, May 19 to August 21 (Fig. 12) ..... *Hyla cinerea*
        dd. Inner envelope small 1.4 to 2.0 mm; outer envelope 4 to 8 mm.
          e. Packets small, seldom over 30 to 40 eggs; vitellus 1.1 to 1.2 mm. Egg-complement, 1,802. Season, May 10 to August 13 (Fig. 18) ..... *Hyla versicolor*
          ee. Packets large, sometimes 100 to 125 eggs; vitellus 0.8 to 1.2 mm, av. 0.95 mm. Egg-complement, 768. Season May 16 to August 21 (Fig. 17) ..... *Hyla femoralis*

cc. Egg packets large, loose, glutinous films, 35 sq. in. to 675 sq. in. (218 to 3,721 sq. cm).

d. Inner envelope absent; vitellus 1.2 to 1.7 mm; egg mass 144 to 675 sq. in. (900 to 3,721 sq. cm) in area, or 12 by 25 inches (30 by 61 cm) in diameter; egg masses amongst brush around the edge of ponds or encircling Pontederia-like vegetation in mid pond. Egg-complement, 10,000 to 20,000. Season, June 1 to July 10 (Fig. 13)..... *Rana catesbeiana*

dd. Inner envelope present, 2.8 to 4.0 mm; vitellus 1.4 to 2.0 mm.

e. Egg mass seldom 1 sq. ft. (35 to 144 sq. in. or 218 to 900 cm) in area or 5 by 7 to 12 inches in diameter; usually around edge of ponds; inner envelope elliptic, pyriform or circular, av. 3.05 mm; vitellus 1.4 to 1.8 mm, mode 1.4 mm, av. 1.5 mm. Egg-complement, 1,451 to 4,000. Season, May 23 to August 21 (Fig. 16)..... *Rana clamitans*

ee. Egg mass over 1 sq. ft. in area (144 to 288 sq. in. or 900 to 1,800 sq. cm) or 12 by 12 inches to 12 by 25 inches in diameter; usually in midpond; inner envelope av. 3.45 mm; vitellus 1.4 to 2.0 mm, mode 1.8 mm, av. 1.7 mm. Egg-complement, 8,000 to 15,000. Season, May 24 to August 21 (Fig. 15)..... *Rana grylio*

aa. Egg mass submerged.

b. Eggs in files or bands.

c. Eggs laid in bands which soon become loose cylinders extending along plant stems or grass blades; vitellus 1.4 to 2.0 mm; envelope 3.8 to 5.6 mm. Egg-complement, 2,332. Season, April 15 or earlier to August 17 (Fig. 5) ..... *Scaphiopus holbrookii*

cc. Eggs in files.

d. Files short (4 to 10 mm in length); 4 to 8 eggs in short bead-like chain or bar or many such files radiating from one focus; vitellus 0.8 to 1.0 mm; tube diameter 1.2 to 1.4 mm. Egg-complement, 610, 766. Season, June 4 to August 21 (Fig. 4)..... *Bufo quercicus*

dd. Files long (several feet in length or often a meter or more long); vitellus 1.0 to 1.4 mm; tube diameter 2.6 to 4.6 mm.

e. Inner tube absent; outer tube 2.8 to 3.4 mm; vitelli crowded in the files; at first in a double row, later more spread out but still crowded; 22 to 24 eggs in 30 mm (1 3/16 inches). Egg-complement, 7,750. Season, April 15 or earlier to August 17 (Fig. 3) ..... *Bufo fowleri*

ee. Inner tube present.

f. 18 to 20 eggs in 30 mm (1 3/16 inches); partition apparent between eggs; inner tube 1.6

to 2.2 mm; outer tube 3.4 to 4.0 mm. Egg-complement, 4,000 to 8,000. Season, April 5 to July 25 (Fig. 1)..... *Bufo americanus*

ff. 7 to 8 eggs in 30 mm (1 3/16 inches; distinct space between eggs; no partition apparent; tube inclined to be slightly emarginate between the eggs; inner tube, 2.2 to 3.4 mm; outer tube, 2.6 to 4.6 mm. Egg-complement, 2,888. Season, April 15 or earlier to August 13 (Fig. 2)..... *Bufo terrestris*

bb. Eggs in lumps.

c. Egg mass a firm regular cluster.

d. Egg mass a sphere 2½ to 4 in. (6.35 to 10 cm) in diameter, containing 2,000 to 3,000 eggs; outer envelope distinct.

e. Eggs black above and white below; inner envelope apparently absent, slightly evident under lens, 3.6 to 5.8 mm; outer envelope 5.2 to 9.4 mm; vitellus 1.8 to 2.4 mm. Egg-complement, 2,000 to 3,000. Season, March 19 to May 1 (Fig. 23)..... *Rana sylvatica*

ee. Eggs brown above and yellow below; inner envelope distinct 2.3 to 3.0 mm; outer envelope 3.6 to 5.0 mm; vitellus 1.6 to 1.9 mm. Egg-complement, 2,000 to 3,000. Season, April 6 to May 18 (Fig. 19)..... *Rana palustris*

dd. Egg mass a plinth.

e. Without inner envelope, complement small, 349 to 474; eggs black above and sulphur or primrose yellow below; eggs further apart than in *R. pipiens* or *R. sphenocephala;* outer envelope 4.9 to 6.9 mm; vitellus 1.5 to 1.8 mm. Egg-complement, 349 to 474. Season, June 21 to August 11 (Fig. 14)..... *Rana virgatipes*

ee. With inner envelope, complement large, 1,000 to 5,000 or more; eggs black above and white below.

f. Vitellus average 2.0 mm (range 1.8 to 2.4 mm); inner envelope 3.1 to 4.4 mm; outer envelope 4.4 to 6.0 mm, mode 5.2 mm, av. 5.3 mm. Egg-complement, 5,000 or more. Season, (?) to August 17 (Fig. 22)..... *Rana aesopus*

ff. Vitellus average 1.7 mm (range 1.4 to 2.0 mm); inner envelope 2.3 to 3.2 mm; outer envelope 3.4 to 6.0 mm. Egg-complement, 1,000 to 5,000.

g. Average outer envelope 5.1 mm (range 4.2 to 6.0 mm, mode 5.0 mm). Egg-complement, 3,500 to 4,500. Season, March 30 to May 15 (Fig. 20)..... *Rana pipiens*

gg. Average outer envelope 3.8 mm (range 3.4 to 5.4 mm, mode 4.0 mm). Egg-complement, 1,054. Season, April 15 or earlier to August 21 (Fig. 21) ..... *Rana sphenocephala*

cc. Egg mass a loose irregular cluster.

d. Egg mass small, less than 1 in. (2.5 cm) in diameter. 20 to 100 eggs in the mass; outer envelopes merged; the one envelope 5.0 to 7.8 mm, rarely 3.0 mm; vitellus 0.9 to 1.2 mm. Egg-complement, 500 to 800. Season, March 19 to May 1 (Fig. 7) ..... *Pseudacris triseriata*

dd. Egg mass an irregular cylinder 1 to 6 in. (2.5 to 15 cm) in length, extending along plant stem or grass blade; envelope, single, 3.8 to 5.6 mm; vitellus 1.4 to 2.0 mm. Egg-complement, 2,332. Season, April 15 or earlier to August 17 (Fig. 5) ..... *Scaphiopus holbrookii*

(See also (c) under (b) Eggs in files or bands.)

ALBERT HAZEN WRIGHT
ANNA ALLEN WRIGHT

CORNELL UNIVERSITY

SOME CHROMOSOME NUMBERS IN NICOTIANA

FOR some years certain genetic experiments involving numerous species and varieties of the genus Nicotiana have been carried on by the Department of Botany of the University of California[1]. In connection with a number of these investigations cytological information has for some time been greatly needed. The writer has had the privilege, under a fellowship grant from the Scandinavian-American Foundation, of spending some months in the laboratory of Professor Otto Rosenberg and of there making some start in securing such information. The present note deals with chromosome numbers of certain more familar species of Nicotiana. The determination of chromosome numbers was preliminary to work upon the cytological phenomena exhibited by hybrids between *N. sylvestris* and varieties of *N. Tabacum*, concerning which another note is in preparation.

The chromosome number of but one species of Nicotiana has as yet been reported in the literature. White[2] and Palm[3] have

[1] The results of these experiments have been published in part in the Univ. Calif. Publ. Botany, Vol. 5, Nos. 1-17.

[2] White, O. E.—AMER. NAT., Vol. 47, p. 206, 1913.

[3] Palm, B. T.—*Bull.* Deli Proefstat. Medan, No. 16, 1922.

found x-24, while Arisz[4] reports x-25 for *N. Tabacum.* The following list is derived from studies of fixed material made by the writer and confirmed in most cases* during the summer of 1922 in California, by Dr. Margaret Mann according to Belling's aceto-carmine method.

x— 9: Langsdorffii* (2 vars.), alata (affinis)*, longiflora;
x—12: sylvestris*, glauca*, suaveolens*, glutinosa*, paniculata*, acuminata;
x—24: Tabacum* (5 vars.), rustica*, (3 vars.), Bigelovii*, nudicaulis.[5]

Three of these counts are open to some question. In the case of *N. alata* homotypic anaphase plates in the fixed material show 10 chromosomes in some cases, although the predominating number in such stages is 9. Similarly, *N. longiflora* can be counted as 9 or 10, but in this case the predominating number is 10. Only a small amount of fixed material of *N. suaveolens* was available and there is some doubt as to whether the number is not larger than 12, possibly 18. Petaloid anthers of *N. Tabacum* var. *calycina* showed numbers of abnormal heterotypic metaphases, involving apparently the presence of both univalent and bivalent chromosomes. Rather striking differences in chromosome size were observed among the various species but in the absence of controlled, duplicate fixations little significance can be given to this evidence.

Further examination of the species the chromosome numbers of which are here reported will be made this summer when material of a number of other species should be available. An effort is being made to grow a still larger collection of Nicotiana species for similar examination in the hope that a combination of genetic and cytological evidence may give a basis for a more rational taxonomic treatment of the genus than has as yet been offered.

THOMAS HARPER GOODSPEED

UNIVERSITY OF STOCKHOLM,
APRIL 17, 1923

[4] Quoted from Palm, p. 16.

[5] For descriptions of the plants grown under these species designations see Setchell, W. A., Univ. Calif. Publ. Botany, Vol. 5, No. 1, 1912.

A REPORT OF A HISTOLOGICAL STUDY OF THE EYES AND GONADS OF MICE TREATED WITH A LIGHT DOSAGE OF X-RAYS

(1) Introduction

The object of this experiment is to determine whether exposure to a light dosage of X-rays over the whole body has any direct effect upon the histological structure of the eyes and of the gonads in adult mice.

Because of the appearance of genetic modifications among the descendants of mice given a similar dose to that described below (Little and Bagg and Bagg and Little now in press) it was thought to be of interest to determine whether or not there was, in animals of the same parent stock, a direct effect of the X-rays of sufficient magnitude to produce histological modifications.

The eyes were chosen for particular study because at least two of the genetic modifications above referred to expressed themselves in the eye, and because Stockard, Guyer and Smith, and others have found that the eye is a common seat for variation under experimental conditions.

The gonads were chosen because in the case of the female, and probably of the male as well, it is certain that the genetic modifications which appeared were at their origin situated in the germ cells. If they are, then, a direct result of or accompanied by gross histological changes, that fact should be easy to recognize.

(2) Material and Methods

A total of 60 adult mice were used, consisting of 17 black males, 17 black females, 11 brown males and 15 brown females. Three of each of these four groups were killed every three days. The exposures to X-rays were made at the Memorial Hospital in New York City, by the courtesy of Dr. H. J. Bagg. Twelve mice placed in a small wire cage were subjected at one time to the X-rays. No filter was used. The distance was 13 inches, and the strength was ten milliamperes, while the time was 12 seconds. This was done each day for five successive days.

Beginning two days after the last treatment, three mice from each group were killed each day, and the eyes and gonads were removed. The eyes were fixed in Zenker's fluid, and stained with

Delafield's hematoxylin and eosin. The lenses were removed after fixation. The gonads were fixed in Bouin's solution and stained with Delafield's hematoxylin and eosin. These preparations were compared with eyes and gonads of unexposed animals similarly prepared.

(3) RESULTS

In the gonads of the X-rayed animals, no changes were observed. In the testes even after the fourteenth day after treatment, active spermatogenesis was found. No degeneration or gross change of any kind could be noted. Likewise in the ovaries eggs were developing normally. No difference was observed in the eyes of the treated and untreated animals.

(4) DISCUSSION AND CONCLUSION

It will thus be seen that no direct effect of the X-ray exposure given to the ancestors of the abnormal individuals reported by Little and Bagg was observed. The possibility of the inheritance of an acquired character or characters being involved in this case is therefore apparently done away with and the whole matter falls much more properly under the head of a direct effect of the germ plasm by the rays themselves.

Of course the possibility that there was on the eye itself a direct effect of a very minute nature still exists, but it seems both from the absence of visible effects and from the regularity of the process of inheritance as described by the investigators referred to, that each genetic modification is the result of a minute and probably direct change in a very limited region of the chromatin material or even in a single gene.

L. H. SNYDER
MARGARET SCHNEIDER
C. C. LITTLE

CARNEGIE INSTITUTION OF WASHINGTON,
COLD SPRING HARBOR, N. Y.